

nature

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Negotiate flexibly, but explain publicly

THE Royal Commission on Environmental Pollution, chaired by Sir Brian Flowers, has just reported on air pollution control (Cmd 6371, HMSO, £1.75) and manages to handle the potentially inflammatory issue of the Alkali Inspectorate in a sensible and pragmatic way. The inspectorate is praised and criticised in about equal amounts, but no major modifications are proposed to its traditional mode of working, namely by collaboration with industry rather than by confrontation.

Air pollution control in Britain is carried out through two very separate administrative machineries. Her Majesty's Alkali and Clean Air Inspectorate is charged with overseeing a limited number of specified processes deemed to be particularly polluting or problematical. (The first of these processes was initially associated with alkali works where, from 1820 on, great clouds of hydrochloric acid gas were released in the manufacture of sodium carbonate from salt—hence the quaint name given to the inspectorate when it was established in 1863.) Inspectors have always worked on the principle that industry should use the "best practicable means" to reduce discharges rather than be held to fixed emission standards. In the course of this, the inspector has played a substantial and unsung role in the rapid transfer of information about anti-pollution technology, and has also been consulted as a matter of course before the installation of new plant. But in spite of continued advances in freeing the air of industrial pollutants, the inspectorate has come in for plenty of criticism. The "best practicable means" approach, its critics allege, can hide all sorts of abuses or agreements to go easy on polluters. The inspectorate has compounded the problem by making inadequate responses to its detractors, partly, no doubt, because inspectors feel themselves constrained by industrial secrecy and partly because staff numbers are only adequate to perform the inspecting job, not to hold colloquia or confrontations with a broader public.

The other means for controlling air pollution is through the local authority, whose environmental health officer must take care of domestic pollution and of those industries not on the inspectorate's list. Some authorities have air pollution units, but many do not, so control is patchy. Relations between the inspectorate and local authorities vary from good to bad (there is no administrative reason why they should ever meet). And for all that some cities have cleaned up the air dramatically over the past 20 years (London now has 70% more sunshine in December than it used to before smokeless zones were introduced), 40% of the nation's premises which were originally planned to be in smoke control areas have still to come under this constraint.

It is the commission's hope that by releasing the inspectorate from the Health and Safety Executive, where it never really belonged, and by subsuming it in a larger Pollution Inspectorate which could deal with other problems such as water pollution and solid waste, the necessary central expertise can be preserved and augmented while making it easier for local authorities to deal with this expertise. This depends on two fairly important assumptions: first, that the major problems in water and solid waste management are amenable to the same sort of negotiation that the inspectorate has evolved for air pollution. There seems no reason why they shouldn't be if a national unit is put together from existing staff in water authorities, central government and so on, but positive results may still be several years off. Second, the Pollution Inspectorate really must take its relations with the public seriously and must hire people appropriately. It cannot simply be allowed to become an expanded Alkali Inspectorate, full of very dedicated experts, employed full time in talking to industry; at least 20% of the staff should be available to talk and listen regularly to the public.

The commission talks of the housewife whose washing has been dirtied by a breakdown in pollution control at a nearby plant and who may be "disconcerted and irritated" by a request to leave a message on the District Alkali Inspector's phone answering machine (inspectors only have part-time secretarial staff!). She might be lucky even to find the right phone number. In the London Phone Directory there is nothing under "Air", "Alkali", "Clean Air", "Clean Air Council" (which advises the government on air pollution), "Her Majesty's Alkali and Clean Air Inspectorate", or "Pollution". A very smart housewife might just possibly alight on "Environment, Department of the", where lurking under a sub-sub-heading is "Noise, Clean Air and Waste". Or she might try her local authority for which, if the phone book is not too out-of-date there could be an entry "Environmental Health Services", but no mention of air pollution. But, of course, if it was smoke from a bus she would have to ring London Transport. And what about smoke from a car?

Nowhere is bureaucracy more anonymous than in the phone book. The first job for a new pollution inspectorate might be to follow the example of some other countries, hire some intelligent telephone-persons and put a number in the directory with the entry

AIR POLLUTION from all sources—
complaints and queries . . .

No charge for this public service . . .





Planning to use science . . .

Vera Rich looks at the Soviet Union's recently published Five Year Plan, in which the prominence usually accorded to science and technology is again apparent.

AS EXPECTED, the Soviet Union's Five Plan for the period 1976-1980, soon to be submitted to the party congress, gives top priority to greater efficiency throughout the economy, specifically in agriculture and industry. But the framework of the Plan as a whole considerably curtails the almost rhetorical aims for science also expressed by the Plan—namely, “the further expansion and deepening of investigations of the laws of nature and society, increasing its contribution to the solution of problems of current importance in the construction of the material and technological basis of communism, accelerating scientific and technical progress and the growth of effectiveness of production, increasing the well-being and culture of the nation, and shaping the communist outlook of the workers”.

The Plan's modest targets for growth, investment and production seem to have been set deliberately low to take into account possible set-backs of the sort that hampered the previous Plan, such as crop failures and inefficiency. The target for national income growth, at 24-28%, is lower than the 28% achieved by the 1971-1975 Plan. Industrial production will rise 35-39% as against 43% previously, while the growth of investment in agriculture is to fall from 60% to 30%. Under the general slogan of improved “quality” and increased production, it is demanded that 90% of industrial growth and all the growth in agriculture and production is to be met by higher labour productivity (as opposed to 80% in the previous plan).

Within this general prospect of harder and better work with no encouragement of major initiatives, which is seen by many as the design of ageing politicians whose active lifetime is unlikely to run to the full term of the Plan, the scientific projects selected for specific mention and encouragement form an interesting cross-section of research. At one end the emphasis on pure and applied mathematics is related in the Plan to the need for the more efficient and extensive computerisation of the economy—growth in the computer and automated equipment sections, at 60-80%, far outstrips the anticipated average for the industrial sector as a whole, and there are calls for cuts in the share of manual labour in production

At the other end there is the use of space research for the study of natural resources, meteorology and navigation, and for “other needs of the national economy”. Nuclear, plasma, solid state and low temperature physics also receive special mention, as do quantum electronics, optics and astronomy; the need for new materials is stressed, especially magnetic semiconductors, superconductors, and “technically valuable” crystals, and the whole physics programme is grouped in the Plan with the need to develop atomic power and other new sources of energy. Indeed, energy-selected industries, including the chemical and petrochemical sectors, will be among the fastest-growing. The 1980 oil target is up 27%, and a rise of 42% in natural gas will enhance the country's potential as an energy exporter.

In a similar manner, the biology programme is linked both to the need to develop new crops and improvements in occupational health. The main aims for agriculture include the development of animal husbandry and the fodder base, but there will be strong pressures for an improvement in agricultural productivity.

To a certain extent, such elaborations of the purposes of research projects in a time of financial cut-backs may be considered as a justification for their continuation. But the problem with Soviet science, which for all the latitude its practitioners possess operates within a rigid system of state planning, is more complex. With only limited resources of labour and capital available, certain projects—the space programme, for example—acquire a favoured position over others as a result of a complex decision-making process in which potential defence applications or international prestige can be important.

Apart from enjoying considerable material benefits, scientists working in these areas usually find few obstructions in the pursuit of their work. It is possible, for example, to order equipment from abroad; and the procedures for obtaining experimental materials are considerably facilitated. As a result, these fields inevitably attract a great majority of the most gifted talent. On the other hand, scientists not working in a “favoured” field find themselves at a considerable disadvantage, at least with respect to the bureaucracy: requisitions for experimental materials

must be made far in advance and often can only be fulfilled by an unofficial exchange of surpluses between laboratories or institutions.

The gap between favoured and non-favoured projects and institutes tends to widen as a result, and once a particular subject, say space research, has acquired a measure of prestige, a major cut-back is more difficult to order, at least in the political life-time of those who first sanctioned it. As for the non-favoured projects, these tend to be staffed by the relatively less able, resulting in a relative decline in performance, so that in certain fields, like precision instruments, a technological short-fall emerges which makes the purchase of required equipment abroad more likely.

Accordingly, although throughout the Soviet Union's history the presumption has been that Soviet science must inevitably outstrip that of the west, the day of total independence of Soviet technology still lies in the future. This is tacitly admitted by the present plan, which includes the purchase from the west of equipment for producing mineral fertilizers and for the development of the oil, gas, paper, cellulose, and “certain other” industries. The stress on the need for computerisation and automation suggests that there will be considerable (if unacknowledged) reliance on western know-how.

One possible solution open to the authorities is the purchase of foreign equipment which can then be studied and reproduced, thus saving research and development costs. The Plan's proposals may therefore be a disguised way of purchasing expertise. During the last few years the Soviet Union has signed a number of agreements on technological cooperation with Western countries which are clearly intended to be of bilateral benefit, and it will be interesting to observe how the fields of cooperation may change in the future as the Soviet Union closes the short-fall gap in this or that branch of technology.

The sensitive relationship between science and industry in the Soviet Union will continue to be revealed over the coming five years. In the short term, because the rigid targets imposed on industry often leave no time for re-tooling or re-equipping, the introduction of some cost-saving and efficient new process may be reflected in the quarterly returns as a falling-off in various industries. The contribution of science to the long term aims of higher productivity and improved efficiency, to be effective, will demand better management and planning of techniques. Yet, oddly, this is a comparatively neglected area in the new Five Year Plan. □

... in public or private?

Colin Norman in Washington examines the arguments which two recent events involving Soviet dissident scientists have helped to regenerate in the USA's scientific community.

ON DECEMBER 12 last year, Sergei A. Kovalev, a Soviet biologist, was convicted of "anti-soviet agitation and propaganda" and sentenced to seven years in prison with hard labour, to be followed by three years of exile. His offence was that he had spoken out in defence of human rights and disseminated outlawed literature. Although he had been in jail for nearly a year waiting trial, Kovalev's plight raised little outcry in the West.

In contrast, early this month Leonid I. Plyushch, a mathematician, was released from a psychiatric institution in which he had been forcibly detained for nearly three years, and allowed to leave the Soviet Union. Plyushch's incarceration, for offences similar to those charged to Kovalev, had provoked many public protests from groups in the West, including scientific organisations and the French Communist Party.

To many observers here, those two events underline a simple and obvious fact—public outcry in the West can play a decisive role in tempering the Soviet government's repression of so-called dissident scientists and intellectuals. And that is precisely why the National Academy of Sciences, the most prestigious scientific organisation in the United States, is being pressured to make more public representations on behalf of individual scientists in the Soviet Union, and to speak out more forcibly in defence of human rights.

Although a few individuals have long urged the Academy to adopt a more aggressive public stand on such matters, the issue has recently received some publicity because of an open dispute between the Academy President, Philip Handler, and Jeremy J. Stone, Director of the Federation of American Scientists (FAS), a liberal organisation with a membership of 7,000 which lobbies for such causes as arms control.

The dispute centres on a brief item in the December issue of the FAS newsletter, in which Stone related three complaints about the Academy's public posture in regard to Soviet dissidents, which he heard directly from beleaguered Soviet scientists. Stone picked up the complaints during a recent visit to the Soviet Union on behalf of the FAS to investigate the problems faced by Soviet scientists who have criticised official policies or who

have applied for exit visas; he printed the complaints as part of a detailed account of his findings.

Handler was incensed by Stone's reporting of the complaints because he claims that it represents a gross distortion of the Academy's actions. He fired off an angry, eight-page letter to the FAS president, Philip Morrison, defending the Academy's record, accusing Stone of an "ugly act", and demanding an apology. Beneath the anger, however, lies a serious issue, namely, what is the best way in which the Academy can use its prestige to seek relief for harassed Soviet scientists, or for scientists in similar predicaments elsewhere in the world?

The Academy operates a number of scientific exchange agreements between the United States and other countries, a fact which gives Academy officials extensive contact with their counterparts in the Soviet Academy of Sciences. In the past few years, those contacts have been used occasionally for private, face-to-face representations by the Academy to seek relief for individual Soviet scientists who have been harassed, dismissed from their jobs or imprisoned for such offences as applying for visas to emigrate to Israel, criticising Soviet policies or speaking up in defence of human rights.

In 1972, for example, during a visit to Washington by M. V. Keldysh, then President of the Soviet Academy of Sciences, Handler and other Academy officials took the opportunity to protest the imposition of hefty exit taxes on scientists who had applied for emigration visas. And early in 1973, during a visit to Moscow, Handler made repre-

sentations on behalf of Benjamin Levich, an eminent Soviet electrochemist who had been fired from his job after applying for a visa to emigrate to Israel. Handler says he took up Levich's case with Keldysh and President Podgorny, and he even asked Keldysh to deliver a letter to Levich awarding him a prize from the American Electrochemical Society and inviting him to the United States to give a lecture. Keldysh refused to accept the letter and accused Handler and the Electrochemical Society of "playing politics".

Handler was therefore particularly incensed by one of the complaints in the FAS newsletter, that during his visit to Moscow he had snubbed Levich by refusing to meet him. Handler said that Levich in fact telephoned him at his hotel an hour after he arrived and invited him to his house. Handler says he refused because such a visit would blunt the impact of his private negotiations on Levich's behalf, a position which is consistent with his view that the Academy should conduct such negotiations in private, without making a public fuss. Similar sentiments are expressed by George S. Hammond, the Academy's Foreign Secretary. Hammond says that "having chosen the quiet diplomacy approach, I think we should stick to it. If we go to Moscow and hold private discussions and then make public statements, it would be a breach of confidence and undermine our position".

Stone, however, takes a different view. "All of my Soviet experience during six visits," he says, "suggests that complaints made in private are often ignored, while those made publicly must be dealt with." As far as Handler's refusal to visit Levich is concerned, Stone notes that because of the publicity which would have attended such a visit, "I have no doubt that Levich was more interested in having Handler visit him while he was in Moscow than in any representations that Handler could have made for him privately".

The Academy has, however, made one very public protest. In September 1973, when the press campaign against Andrei Sakharov was at its height, Handler sent a sharply worded protest to Keldysh, warning that unless harassment of Sakharov ceased, scientific co-operation between the United States and the Soviet Union could be jeopardised. Shortly thereafter, the press campaign abruptly stopped. Though Sakharov has, of course, come in for more criticism and intimidation since, it is widely believed that he is no longer in imminent personal danger. According to Pavel Litvinov, a Soviet physicist who was exiled to Siberia after protesting against the Russian invasion of



Jeremy Stone, FAS Director

Czechoslovakia, and who is now living in the United States, "the representation by the National Academy of Sciences in the case of Sakharov was effective. Of that I am sure". He added that "in every case when the Soviet bureaucracy has given in, it has been done by open pressure". Handler maintains that the public protest on Sakharov's behalf was made in response to a critical situation—the fear that Sakharov was about to be arrested and tried for treason. He suggested that such an approach should not be used in less dramatic situations.

Stone believes, however, that the initial success of the Sakharov protest should convince the Academy that occasional public protests would greatly strengthen its hand in private negotiations. And Lipman Bers, an Academy member and President of the American Mathematical Association who has made representations on behalf of beleaguered scientists in several countries, notes that "my impression and experience is that so-called quiet diplomacy and public protests reinforce each other". Similarly, Harrison Brown, a former Foreign Secretary of the National Academy of Sciences who played a key role in the Academy's protest over Sakharov, suggests that "if anything, the Academy has erred on the side of not doing enough publicly", though he adds that he believes that the private approach "has tempered Soviet actions" and notes that "it is very difficult to get a proper balance between public and private approaches".

Stone, meanwhile, is working to get the prestige of Academy members behind some public protests to be launched by the FAS on behalf of dissident Soviet scientists. Last month, he sent a letter to every member of the Academy, asking whether they would be willing to lend their support to petitions "for scientists being denied the right to function as scientists". He said last week that he anticipates a positive response from about 25% of the Academy's members. In addition, he has circulated a petition among physicist members of the National Academy of Sciences asking for their support for Andrei Tverdokhlebov, a physicist who was arrested nine months ago for allegedly disseminating false material and whose trial is imminent. A very high proportion has already responded. A petition has also been mailed to some 20,000 biologists in support of Kovalev. Stone also attempted, unsuccessfully, to ensure that international observers would be allowed at Kovalev's trial.

The Academy is therefore under some pressure to take a more aggressive, public stand in support of dissident Soviet scientists. It should be noted, however, that such a move would draw

strong criticism from the State Department and other government agencies since it would seem to run counter to the spirit of detente. The Administration would much prefer a quasi-government body like the Academy to work behind the scenes, leaving the public protesting to private organisations like the FAS.

The issue of how learned societies should handle relations with their

counterparts in the Soviet Union has, of course, also been aired in other countries. In the UK scientists debated the subject on television in 1973, and in 1974 the Council of the Royal Society considered the issue following an initiative by Professor John Ziman. The continuing low profile of the society makes it reasonable to assume that proposals for a more public stance were turned down. □

An appeal for help

THE following quotations are taken from a letter written by Valentin F. Turchin, a Soviet mathematician who was fired from his job in July 1974 after he had made a public statement in defence of Andrei Sakharov. Turchin, who is chairman of the Soviet group of Amnesty International, has been out of work for 18 months, and has applied for permission to visit the United States to work at Columbia University. He was informed on December 15 that his application had been denied. The letter was received on January 13 by Jeremy Stone, the FAS Director. Turchin says that he wants it to be discussed by the scientific community in the West. It will eventually be published in full by the Khronika Press in New York.

Turchin begins by describing the harassment and the trial last month of Sergei Kovalev. Kovalev, an eminent biologist, was given the maximum sentence of 7 years' imprisonment with hard labour and a further three years of exile within the Soviet Union for "anti-Soviet agitation and propaganda". Noting that, together with Sakharov, he had appealed for help for Kovalev from Western scientists, Turchin states that "there was no response deserving to be mentioned and I don't know whether there was any response at all . . . No action was made which could have attracted serious public attention and influenced Soviet authorities. The world scientific community betrayed Kovalev".

He continues: "You are very proud, my dear colleagues, that you separate science from what you call politics. You do not go in for politics, you say. Neither do we. Dissidents in the Soviet Union do not go in for politics: they struggle for air. What you are separating science from is not politics but mere decency. And in fact, it is not separation, but a reversal, changing of the sign. For whatever you think, you are not neutral in the conflict between totalitarianism and freedom. You actively cooperate with totalitarianism, support it . . .

"People of science are intrinsic enemies of totalitarianism, because they professionally need intellectual freedom. The core of the Soviet dissidents consists mainly of scientists. But the state presents to the scientist a dilemma; either to support totalitarianism, to lie and betray comrades, or to challenge it to some extent and pay in proportion, by professional losses up to the point of losing work and freedom. The Western scientific community helps to conduct this policy by fully accepting the totalitarian rules of the game in scientific contacts with the USSR and the satellite countries. One example will suffice: did you ever turn back a Soviet delegation because the scientists you had invited were not included [because they were] politically unreliable? Politically reliable people, that is those who help strangle the recalcitrant, are allowed by the Soviet authorities to come out on the international scene. You give your sanction to this selection . . .

"Why not demand, for example, that a small proportion of those who participate in scientific exchange—say, one in ten—must be the other side's choice, and if not, then firmly refuse to cooperate? Scientists hold powerful levers of influence on totalitarian countries. Why do they not use them to save a colleague from imprisonment? . . .

"The detente is necessary, I'm completely for the detente. But in the absence of strong public pressure for human rights all over the world the detente will automatically lead to proliferation of totalitarianism. The Helsinki agreement reveals a typical pattern: the West makes real concessions in exchange for imaginary ones from the East. After Helsinki, the situation with human rights in the USSR has become only worse . . . The proponents of the Helsinki agreement argued that it would provide the grounds for exerting pressure on the USSR for exchange of people and ideas. But what is the use of the grounds if there is no desire to exert pressure? . . ."

CANADA

Royal Society may acquire an expanded role

The ways in which Canada's Royal Society might have a broadened role in the country's scientific affairs is becoming a matter of some controversy. From Ottawa, David Spurgeon reviews the main issues, which focus chiefly on the question of relations with the government.

THE contract for \$75,000 over a 12-month period which was signed not long ago between the Canadian federal government and the Academy of Sciences of the Royal Society of Canada will enable the academy to undertake a study to identify specific missions in the natural and human sciences that may be of value to the government, and to recommend policies and procedures for carrying them out. Thus, apart from allowing the academy to engage an executive director half-time—he is Donald Hurst, former head of the Atomic Energy Control Board of Canada, the country's regulatory agency in nuclear affairs—the contract may give it a broadened role in Canada's scientific affairs, the implications of which do not delight all its members.

A number of initiatives designed to make the Royal Society more influential in scientific affairs has been taken in recent years. Five years ago, a committee set up by the Society's old Section Three (now the Academy of Sciences) to study how this could be done became a committee of the Society as a whole within months. Now there are three component academies in the Society, the other two being those of the social sciences and humanities (one for the Anglophone section, the other for the French).

One of the reasons some have felt the need of a stronger role for the Royal Society in science was expressed by the geologist J. Tuzo Wilson when he was president in 1973. In an article in *Science Forum* he said that the Society believed that Canada needed "an active and independent academy, not so much to advise government, but to promulgate informed opinions and to lead open discussions of important issues that will aid governments in making their decisions".

Dr Wilson stressed that, unlike the various bodies the government had established for similar purposes, the Royal Society was not a political body. He suggested that its independence, together with its access to leading scientists in many fields, could provide the alternative in scientific advice that

the country needed. It was his view that the whole direction of Canadian science was in the hands of civil servants and members of councils who are appointed by ministers, and he claimed that the whole direction of science had been "politicised".

Not all Society members have agreed on the role the body should take, however. Although some, like Wilson, thought a detached role (in which the Society would make studies in specific areas) was best, others thought it should be closer to the government and play a more official advisory role.

Part of the Society's expanded role may be in international scientific affairs. In other countries, contacts with international scientific unions are usually made through non-governmental bodies, but in Canada the National Research Council has usually provided the point of contact. Some think this anomalous. For the social sciences, the humanities and engineering, Canada has non-governmental organisations that can act as adhering bodies to the international unions: the Social Science Research Council, the Humanities Research Council, and the Canadian Council of Professional Engineers. But there are no corresponding non-governmental bodies for international contact in the medical and natural sciences.

Some members of the Royal Society believe this is a role the Society should play, as academies do in other countries. But the problem of how this could be done remains because of the large number of professional organisations in different disciplines in Canada and the number of constituent bodies of the international scientific unions. One suggestion is that the Society should play the role of clearing house for the appropriate national professional organisations.

A certain amount of scepticism usually accompanies changes which the Royal Society of Canada makes, mainly because many scientists have become accustomed to thinking of the organisation as a self-congratulatory old boys' club. In spite of this, the 'club' counts among its members many of the country's most distinguished scientists (and lately even a few engineers), and it has been stirring itself in recent years to play a more important public role—for example by organising seminars on such topics as the energy problem. The outcome of this latest move will thus be watched with interest. □

BRITAIN

University pay grumbles remain

Last year British university teachers were involved in 'industrial action' before finally resolving their dispute with the government over salary increases. The government has since introduced its pay policy. This month many academics will receive for the first time the cost of living element of their 1975 salaries settlement. David Walker reports on the discontent that still prevails.

THE Association of University Teachers (AUT), the university authorities and the British government have agreed that the pay code maximum of £6 a week should be added to all academic salaries under £8,500 a year, and the whole package backdated to October. The final figures for the delayed settlement—the basic element was determined by an arbitration body as long ago as May last year—show that junior lecturers have probably benefited most and now start their teaching careers on nearly £3,200 a year. Some staff, who will no doubt bear in mind Mr Healey's recent warning about "confetti money", have increased their annual salaries by £1,000 in the space of 12 months. And one professor of politics told the world on television before Christmas that he was "aggressively satisfied" with his standard of living.

Under the settlement the starting point for all university academic staff, the foot of the lecturer grade, is £3,174 a year rising through 16 incremental points to £6,446. The scale for senior lecturers and readers will now run from £6,234 to £7,742, and the professorial minimum salary will be just below £7,900. Professors outside the clinical subjects will be on an average of over £9,200, although few of them got the £6 a week element or at least got only enough of it to take them to the £8,500 salaries ceiling. The settlement will run for the duration of the Labour government's incomes policy and Mr Laurie Sapper, the general secretary of the AUT, claims they have extracted from the government a firm promise to revise the scales of payment as soon as economic circumstances permit.

In spite of that, there is a lingering mood of dissatisfaction in those senior common rooms where salaries get discussed—and it is a sign of the times that money now gets talked about in public. Those university teachers who take an active part in their trade union (and a key test of their 'militancy' will come in March when they will vote on whether the AUT should affiliate to

the Trades Union Congress, TUC) are still disgruntled. One of the major issues they still consider unresolved is the question of comparisons between their salaries and those paid to polytechnic lecturers and heads of departments.

Straight comparisons between rewards in the two sectors of British higher education are notoriously difficult to make on account of different opportunities for promotion, the numbers of staff, on relative salary points and the balance in teachers' time between teaching and research. Yet they are made, and the AUT officially considers that many university people are comparatively underpaid.

In addition the bi-annual conference of the AUT last December showed that

the government's handling of negotiations on pay during the past year has left a legacy of bitterness. There is talk of "hatred" for the universities in the Department of Education and Science, discrimination in favour of the polytechnics, and—most disturbing—of a Civil Service plot to do down the universities.

The worst of these suspicions are likely to clear in the coming year, however. Mr Fred Mulley, the Secretary for Education, is a genial figure who attracts none of the opprobrium heaped on his predecessor, Mr Reg Prentice. Although the university teachers are likely to vote in favour of joining the TUC, it is unlikely that a moderate body like the AUT will do much to steel its arms for militant action on

the issue of salaries.

But action is promised on two fronts that could have important longer term effects on the structure of the profession. The AUT is planning to press for a revision of the ratios of senior to junior staff, on the grounds that there is a serious promotions log-jam, with a great build-up of those on the upper points of the lecturer and senior lecturer scales with little or no prospect of advancement. On another front the AUT is drawing up new salary claims to be presented to the government on the basis of comparison with the Civil Service. This marks an unwelcome development in the eyes of those who fear that the drawing of such analogies will speed the process of bringing universities into full state control. □

FORTY-EIGHT years ago I published the first of a series of papers on the exponential growth of colonies of duckweed and the circumstances under which exponential growth curves became sigmoid. I was following the fashion at that time. Raymond Pearl was doing elegant mathematical analyses of the growth rates of human populations, and Alfred Lotka's brilliant *Elements of Physical Biology* (surely one of the most seminal books on mathematical biology ever written) came into my hands when I was a third year student, just 50 years ago. It is a book which gave, as long ago as that, a masterly treatment of the conditions for equilibrium in biological systems.

So it is with a sensation of nostalgia that I have followed the publications sponsored by the Club of Rome and supervised by Dennis Meadows. It is touching to watch the rediscovery of exponentials, as though Pearl and Lotka had never lived, and ironical to note how much more credible those writers were, working with a slide rule and a little algebra, than Meadows and his colleagues are, armed with a computer. With these thoughts in mind I turned with hope rather than anticipation to the third volume of studies sponsored by the Club of Rome (*Dynamics of Growth in a Finite World*. Pp. 637; Wright Allen; Cambridge, Massachusetts, 1975). Have the authors, as I hoped, at last recognised the fundamental weakness of computer simulations in which the critical parameters—the adaptation of social values and norms to environmental change—have to be omitted because they cannot be quantified?

If this third volume does nothing else, it illustrates the phenomenon of exponential growth. The first volume weighs 170 g and cost \$2.75; the

second weighs 530 g and cost \$18; the third weighs about 900 g and costs (in sterling) the equivalent of \$37. If one makes the assumptions which are the theme of *The Limits to Growth*, the next volume, due in a couple of years,

Exponentials again



ERIC ASHBY

will weigh $1\frac{1}{2}$ kg and cost about \$60, and the one after that 5 kg, perhaps.

Of course nobody in his senses makes such assumptions, but then neither does he make similar ones about the exponential increase of pollution in nations which have NEPA or the EEC Declaration on the Environment or the Control of Pollution Act; yet a section in chapter six of this book does make precisely this assumption, and the authors reach a conclusion which I think (without immodesty) I could have reached without a computer: "... as long as there is exponential growth in the generation of pollution, ameliorative measures ultimately do little to prevent the model from exhibiting unacceptable levels of pollution

damage" (page 477).

We owe a debt to Meadows and his colleagues for dispelling the utopian mirage: that material standards of living will continue to go up and up, until Indian peasants enjoy the comforts and amenities now enjoyed by the middle classes in Europe. But they do a disservice to their own cause when they imply that their computer simulations can be used as a guide to policy making. A glance at the present state of the world is enough to demonstrate that this naive approach (even though it does include an element of social feedback through the price mechanism) diverts attention from the much higher priorities in policy making. Long before we are starved of resources or smothered in excess population or choked by pollution we are in for a series of grave geopolitical confrontations, to be resolved either by war or by subjection for nations which need resources to conditions imposed by nations which own resources.

The world's future in the short run depends on how these confrontations are handled. The techniques for handling them are not in the hard sciences (though they will buy time) nor in econometrics (which has a dubious record of predictiveness). They are in social anthropology (to understand the nations which may inherit power), in ethology (to understand our own social behaviour under stress), in political science (to help us to devise political systems to match the complexity of post-industrial society). These are the fields which need massive support for research and development. I think sometimes that Britain suffers from a semantic anomaly: that *Wissenschaft* in Germany and *Nauk* in the USSR cover a much wider spectrum of disciplines than our word *Science*.

news and views

Search for organic superconductors

from A. D. Yoffe

A THEORETICAL paper published in 1964 by W. A. Little (*Phys. Rev.*, **134A**, 1416) dealt with the possibility of high temperature superconductivity particularly in solids formed from long chain organic molecules. The use of the term "high temperature" is of course a little ambiguous, but a realistic interpretation would put this in the vicinity of liquid nitrogen temperature (77 K) or higher. This theory was based on a particular kind of electron-phonon (vibrational) coupling and it naturally created considerable interest and excitement. First, there were the biological implications and second, there was the vision of a relatively cheap high temperature superconductor for both scientific and industrial use. It should be said that the paper was also greeted with a good deal of criticism and scepticism. This led to a hunt for organic-type solids which would be metals at room temperature and which would remain metals even when cooled to temperatures below that of liquid helium (4 K). This is not an easy problem to solve since pretty well all organic solids are insulators or semiconductors. One exception is of course graphite and the intercalate compounds formed from graphite with, say, alkali metal atoms such as potassium. They are metals in this sense, but they hardly qualify as organic solids.

Crystals of the organic charge transfer complexes such as TTF-TCNQ (tetrathiofulvene - tetracyanoquinodimethane) which are extremely anisotropic in their physical properties, do behave as metals with a conductivity in the region of $1,000 \text{ ohm}^{-1} \text{ cm}^{-1}$ at room temperature when this is measured along one of the crystal axes. This is the direction along which the molecules are stacked. Further evidence for the metallic nature along this direction came from optical reflectivity experiments using polarised light. When the electric vector of the incident light is parallel to the direction of the stack of molecules (good conductivity direction) then typical metallic free carrier reflectivity is found. When the crystals are cooled below liquid nitrogen temperatures

(round 60 K), however, the electrical conductivity begins to fall, and it seems that the solid changes to a small band gap semiconductor. The solid undergoes what is currently termed a Peierls transition, proposed for one-dimensional metals. In 1973 Heeger and his colleagues at the University of Pennsylvania published some rather startling results on the electrical conductivity for some of their crystals of TTF-TCNQ in which they found the conductivity rose to very high values approaching that for copper, just before the transformation temperature to the semiconducting state. There was talk of superconducting fluctuations and there can be little doubt that these experimental findings created quite a stir and led to the renewed interest in this and similar systems over the past few years.

TTF-TCNQ belongs to the class of solids built up from chain-like structures which are loosely called "one-dimensional conductors". Other examples are $\text{K}_2\text{Pt}(\text{CN})_4\cdot\text{Br}_{0.3}\cdot 3\text{H}_2\text{O}$ (commonly abbreviated as KCP(Br)) and the sulphur nitrogen polymer, usually called (SN)_x polymer. This polymer consists of helical chains of sulphur and nitrogen bound together in the crystal by relatively weak van der Waal's forces. The crystals have the appearance of brass, and in 1975 the IBM group (Greene, Street and Suter, *Phys. Rev. Lett.*, **34**, 577; 1975) reported that crystals of this material did in fact become superconducting, but at a relatively low temperature round 0.3 K. This is the first polymer to be shown to be a superconductor and although the result could be likened to a damp squib when we consider the magnitude of the transition temperature, it is important and provides the hope of synthesising other polymers which have higher superconducting transition temperatures.

Many research groups throughout the world with strong chemical support are at present attempting to synthesise such organic solids which behave as metals at all temperatures and which do not display the unfortunate characteristic of TTF-TCNQ at low temperatures. One interesting development has

been the synthesis of the charge transfer complex HMTSF-TCNQ (hexamethylene tetraselenafulvalinium-tetracyanoquinodimethane) which remains metallic below one degree Kelvin, although it does not become superconducting (Block, *et al.*, *Phys. Rev. Lett.*, **34**, 1561; 1975). Another and rather better example is the organic complex radical anion salt 1,2-di (N-ethyl-4 pyridinium) ethylene²⁺ (7,7,8,8 - tetracyanoquinodimethane)²⁻ discussed in this issue of *Nature* (page 201) by Ashwell, Eley and Willis.

A good deal of important physics and chemistry is coming out of this work on solids composed of chains of one kind or another. These crystals are extremely anisotropic in their physical properties, and we find that we are concerned with phenomena such as Peierls distortions, Kohn anomalies, soft phonon modes, superlattice formation, charge density waves and superconductivity. It is clear however that the real goal is the synthesis of organic solids which will become superconducting at reasonable temperatures. The experiments discussed above point the way in which future work might develop. □



A hundred years ago

THE Ladies' Classes at University College, London, began on Monday last the second term of their eighth session. There was a slight decline in the number of students for the session 1874-75, but the first term of the session 1875-76 showed a considerable advance beyond the highest success hitherto attained. In the Michaelmas term, 1874-75, the whole number of individual students was 199; in the Michaelmas term, 1875-76, just elapsed, the number of individual students was 265. The whole number of tickets taken in Michaelmas term, 1874-75, was 257; in the same term of 1875-76 it was 367. from *Nature*, 13, 235; January 20, 1876

HeLa takes over

by Sandy Grimwade

ALTHOUGH it was suggested in 1967 by Gartler (*Natn. Cancer Inst. Monogr.*, **26**, 167) on the basis of isoenzyme patterns that not all cell lines in common use were what they purported to be, it was not until last year that cytogenetic studies by Nelson-Rees *et al.* (*Science*, **184**, 1093; 1974) demonstrated conclusively the extent of contamination. Gartler showed that many commonly used cell lines, supposedly of Caucasian origin, contained the A-type isoenzyme of glucose-6-phosphate dehydrogenase (G6PD), which is in fact found only in approximately 30% of the Negro race. One cell line which legitimately contains G6PD type A is the HeLa line, the oldest and most widely used of all cell cultures, which was established in the early 1950s from the cervical carcinoma of a Negro woman in Baltimore named Henrietta Lacks. The warning attached to that finding was that many cell lines could be contaminated with HeLa.

While identification of a single isoenzyme marker left room for doubt, the advent of chromosome-banding techniques by the trypsin-Giemsa and quinacrine mustard methods showed far more specific markers. Examination of HeLa cell chromosomes by these methods shows four unusual chromosomes formed by fusion of portions of various normal chromosomes. Although there remains some disagreement between various groups of cytogeneticists about exactly which chromosomes have fused to form the markers, there is no divergence concerning the occurrence and appearance of the markers in HeLa cells.

In 1974 Nelson-Rees *et al.* reported that several commonly used cell lines from various human sources contained HeLa marker chromosomes as well as the type A G6PD. A third indication consistent with the claim that these were HeLa cells was that no Y chromosome was present. Several of the cell lines were presumed to be of male origin, and should therefore have shown Y chromosomes, whereas HeLa cells are originally female.

The extent of the HeLa take-over of cell cultures is further documented in this issue of *Nature* (page 211) in a paper from Lavappa, Macy and Shannon from the American Type Culture Collection (ATCC). The ATCC, which preserves cultures of every imaginable microorganism and animal cell, distributes its cell lines to laboratories both in the USA and elsewhere. Several thousand samples of cell cultures are sold on a non-profit basis annually. Shortly after the introduction of the

isoenzyme technique, the screening of the entire stock of human cell lines for type A G6PD was initiated and in the 1972 ATCC Registry of Animal Cell Lines 27 out of 56 lines are flagged as containing this marker isoenzyme. The paper by Lavappa *et al.* presents the first results from a cytogenetic screening programme recently initiated using the newly-available chromosome banding technique. Detailed chromosome analysis of several cell lines reveals that five of the lines, all of which bear the type A G6PD, have HeLa marker chromosomes. Since submission of the paper, several other lines have been tested, and so far all the lines with the suspect G6PD isoenzyme have also been found to bear the HeLa markers. Not all these lines have all four markers, however. Detroit 6 cells, for example, lack HeLa marker (HM) 1, but contain two copies of HM2, 3-4 copies of HM3 and show some variability in HM4. These variations are thought to have arisen by somatic cell hybridisation between the original cell line and contaminating HeLa cells. There is also the remote possibility that these markers have arisen spontaneously in more than one cell line.

The results of the ATCC survey and of several other investigations of cell lines in general use have been combined in an extensive table in a recent *Science* paper by Nelson-Rees and Flandermeyer (*Science*, **191**, 96-98; 1976). In addition to the criteria already mentioned, phosphoglucomutase isoenzyme and HLA antigen data are included. In all, more than 70 cell lines with HeLa characteristics are listed.

These results should give rise to some reappraisal of results in the many laboratories where these cultures are being used. Investigators who think they are working with liver or bone marrow, for example, may find that they have been dealing with Henrietta Lacks' extraordinary carcinoma all along.

It is interesting to speculate on the reasons for the virulence of HeLa cells and the origins of the widespread contamination. It seems reasonable to say that HeLa cells being the oldest line in existence have been subjected to the maximum selective pressure. HeLa cells have been cultured an untold number of times. Those in the ATCC have probably been passaged more than 400 times since their initial isolation in the relatively unrefined culture conditions of 1951 and are therefore perfectly adapted to laboratory conditions. The chromosome studies at the

ATCC were carried out on cells passaged only once or twice from the frozen seed stock. As the cell lines now shown to be contaminated with HeLa were mostly frozen and deposited at the ATCC in the early 1960s, often after several hundred passages, the contaminations probably occurred long before their deposition at the ATCC in laboratories where more than one cell line was being handled. The basic biochemical or genetic reason for the virulence of the HeLa cells which would account for their ability to take over any cell culture remains a mystery however.

Although several of its human cell lines are now shown to be contaminated with HeLa, and up to half of them are suspect, the ATCC intends to continue carrying these lines in its catalogues. Despite the fact that the cell lines are similar in their chromosomes, they retain individual characteristics, such as growth rate and virus susceptibility, worth preserving. □

Nova Cygni 1975

from P. J. Andrews

NOVA Cygni 1975, first reported by Honda, was independently discovered by hundreds of astronomers as a bright star in the constellation Cygnus on the evening of August 29. It subsequently became the brightest nova seen since Nova Puppis 1942. First indications that the nova was unusual came when a search for the prenova on the Palomar Sky Survey plates showed no star at the nova's position. The rise in brightness, 18 mag, was greater than any previously known; in fact it led to the suggestion, subsequently refuted, that the object was a supernova.

The most widely held theory for the nova phenomenon is that the prenova is a close binary system consisting of a red dwarf, which is filling its Roche Lobe, spilling matter over towards a white dwarf companion. The period of the binary is shorter for systems containing fainter, cooler red dwarfs. The nova outburst is believed to be due to the flow of material from the red star passing by way of an accretion disk to the white dwarf's atmosphere where it is compressed and heated by the gravitational field until it reaches the ignition temperature for hydrogen burning reactions. Once ignition occurs there is a thermonuclear runaway generating the necessary energy to produce the very hot expanding shell of gas which is seen as the nova eruption.

The development of the nova is the evolution of this expanding region. In general the development is complex with several different systems of absorp-

tion lines present in the spectrum which are due to gas ejection continuing after light maximum. For some novae there are great changes in this outflow which are manifested in dramatic changes in the light curve and spectrum.

Nova Cygni, being bright and easily accessible to all northern observatories, was expected to throw light on the various rival theories for different aspects of the nova phenomenon. Unfortunately it does not seem to be, in any sense, a typical nova.

From comparison of the strengths of the interstellar lines in the early spectrum of the nova with those in the supergiant 55 Cygni, Tomkin, Woodman and Lambert (*Astron. Astrophys.*, in the press) deduced a distance for the nova of somewhat greater than 1 kpc and an intrinsic visual luminosity at maximum of about -10 mag. A similar brightness was obtained from the rate of decline in brightness shortly after maximum by Lindegren and Lindgren (*Nature*, **258**, 501; 1975). This intrinsic luminosity is the highest known for a galactic nova, and the speed of decline is the fastest.

The spectrum during this rapid decline also changes very rapidly and does not show all the 'normal' absorption systems seen in typical novae spectra. Possibly this indicates that for Nova Cygni an instantaneous ejection model is more applicable than the continued ejection models which seem better suited to most novae.

While the decline from maximum light has, compared with many novae, been remarkably smooth, the confirmation (Marrucci, Messi, Natali and Rossi, page 186 of this issue of *Nature*) of a small periodic variation in brightness with a period of some 3 h suggests the possibility that some form of modulation of the nova's brightness is being caused by the orbital motion of the binary.

The observation of coronal lines in the near infrared by Grasdalen and Joyce (see page 187) indicates the presence of a very high temperature ($\sim 10^6$ K) region where apparently the degree of ionisation is still increasing. They suggest that there is the possibility of determining the physical conditions and abundances in this region which is presumably situated close to the site of the nova explosion. Abundance determination should also be possible for a series of high dispersion spectra obtained at the Royal Greenwich Observatory by Stickland within 8 h of discovery. A preliminary analysis by the author indicates that the strengths of the CII, OII and especially NII lines are greater than would be expected suggesting that these elements have greater than normal abundance. This would agree with the theoretical pre-

dictions of Starrfield, *et al.* (*Astrophys. J.*, **176**, 169; 1972) who indicated that these elements will be enhanced in this way by the nova mechanism discussed above.

Shortly after maximum light the spectrum became dominated by emission lines which became progressively stronger relative to the continuum as the nebular stage was approached. At the same time structure at the top of each emission line became apparent. Thus structure, initially seen as four peaks at each emission line, has subsequently developed so that by the end of September up to eleven peaks were seen and measured in each of several different spectral lines. The derived velocities were constant for each peak, where present, for all the spectral lines measured. The expanding nebula is thus strongly lobed in a very complex manner which together with observations of circular polarisation in H α and H β by Kemp and Rudy (*IAU Circ.* 2837) perhaps supports Mustel's ideas that strong magnetic fields are present in novae.

There is, no doubt, an enormous amount of observational data on Nova Cygni accumulating. The first results are only now starting to appear, but Nova Cygni seems so far to have posed more problems than it has solved. $\square$

Expression of eukaryotic genes in prokaryotes

from Giorgio Bernardi

MANY of the hopes, and some of the fears, associated with future developments in the field of genetic engineering rely upon the possibility of transcribing and translating eukaryotic genes in prokaryotic systems. It is not yet clear, however, whether the ambitious goal of converting bacteria into factories for the synthesis of medically, agriculturally and industrially important proteins of eukaryotic origin can be achieved without some additional advances in our technology.

Following the pioneering work of Berg and his colleagues at Stanford, restriction endonuclease-generated DNA fragments from various sources have been cloned in *E. coli* by linking them to a plasmid (or a phage) replicon, and then introducing the composite molecules into bacterial cells by transformation. After having been first used to introduce segments of other bacterial plasmids and phage DNA into bacteria (see for example Chang and Cohen, *Proc. natn. Acad. Sci. U.S.A.*,

71, 1030; 1974; Herschfield *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3455; 1974; Tanaka and Weisblum, *J. Bact.*, **121**, 354; 1975), this method has been used to clone fragments of eukaryotic DNA, such as *Xenopus laevis* amplified rDNA (Morrow *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1743; 1974), *Drosophila melanogaster* DNA (Wensink *et al.*, *Cell*, **3**, 315; 1974; Thomas *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4579; 1974; Glover *et al.*, *Cell*, **5**, 149; 1975; Tanaka *et al.*, *Biochemistry*, **14**, 2064; 1975) and sea urchin histone genes (Kedes *et al.*, *Nature*, **225**, 535; 1975; Birnstiel *et al.*, *Xth FEBS Meeting*, **38**, 3; 1975).

In order to study the expression of eukaryotic genes in the prokaryotic host, advantage has been taken of the fact that plasmids segregating into minicells (which are devoid of bacterial chromosomal DNA) are capable of plasmid-specific synthesis of functional gene products (Frazer and Curtiss, *J. Bact.*, **115**, 615; 1973; Van Embden and Cohen, *J. Bact.*, **116**, 699; 1973). For both rDNA and histone genes, it has been shown (Morrow *et al.*, *op. cit.*; Kedes *et al.*, *op. cit.*) that transcription takes place in minicells; the fidelity of transcription has not, however, been demonstrated in either case.

A novel approach to this problem has been tried by Chang, Lansman, Clayton and Cohen (*Cell*, **6**, 231; 1975) by looking at the expression of mouse mitochondrial DNA in minicells. This genome has a size of only 10×10^6 and can be cloned in its totality, thus providing an opportunity for studying transcription and translation of a complete eukaryotic genome in a prokaryotic system, in contrast to previous studies which only involved genome fragments.

Covalently closed, circular mtDNA was partially digested with *EcoRI* restriction endonuclease, (in order to obtain linear molecules broken at only one of the two *EcoRI* sites on mouse mtDNA), and annealed with *EcoRI*-digested pSC101 plasmid DNA; after ligation, the DNA mixture was used to transform *E. coli* cells which were grown in the presence of tetracycline to allow selection of cells transformed by the antibiotic resistance-carrying plasmid. The total closed, circular DNA population was then isolated from transformed cells and fractionated by sucrose gradient centrifugation. The 44S DNA molecules, corresponding to a molecular weight of 16×10^6 , and therefore to the sum of mtDNA plus plasmid DNA, were amplified by a second cycle of transformation. Four different chimaeric molecules were recognised by heteroduplex analysis and by degradation with *HindIII*, a restriction enzyme having three sites on mtDNA and one (practically coinci-

dent with the *EcoRI* site) on pSC101. These molecules differed in the mtDNA *EcoRI* site used for plasmid insertion and in the relative orientation of plasmid and mtDNA.

MtDNA was then isolated from *EcoRI* digests of chimaeric plasmids by centrifugation in CsCl-netropsin density gradients, and separated into its heavy and light strand by centrifugation in alkaline CsCl. RNA labelled for 30 min with ³H-uridine, obtained from minicells carrying chimaeric plasmids showed a continuous size distribution, as if unstable messenger-like RNA was being isolated in different stages of synthesis and degradation. In addition, most of the 30 min- and 3 min-labelled RNA hybridised to the L-strand, whereas little, if any, hybridised to the H-strand. Since the preferential transcription of the L-strand is not influenced by the relative orientation of pSC101 and mtDNA in the chimaeric molecules, it is improbable that transcription of the L-strand depended on a pSC101 promoter and read through at the mtDNA-pSC101 junction. Thus, one or more signals located within the L-strand are, indeed, recognised by the *E. coli* RNA polymerase. This does not, however, establish any structural identity between these sites and the promoter regions used inside functional mitochondria. In fact, as stressed by the authors, the opposite view is favoured by two lines of evidence. *In vivo*, mitochondrial rRNAs and most tRNAs and mRNAs are transcribed from the H-strand, only three tRNAs and one mRNA originating from the L-strand (Attardi *et al.*, in *The Biogenesis of Mitochondria*, 9, edit. by Kroon and Saccone, Academic Press, 1974). *In vitro*, mitochondrial RNA polymerase preferentially transcribes the H-strand (Wu and Dawid, *J. biol. Chem.*, **249**, 4412; 1974), whereas *E. coli* RNA polymerase preferentially transcribes the L-strand (Tabak and Borst, *Biochim. biophys. Acta* **217**, 356; 1970; Dawid, *Devl Biol.*, **29**, 139; 1972).

The total incorporation of ³H-leucine into protein in minicells carrying the four types of chimaeric plasmids was found to be 2-4 times higher than that obtained from minicells containing pSC101 alone. The label was present in four polypeptides ranging in molecular weight from 2,500 to 5,000; four of the five pSC101-determined proteins were also seen. The protein synthetic pattern observed suggests that translation occurred from RNA transcripts of mtDNA; it differed, however, from that observed in mouse mitochondria, in which case seven proteins ranging in molecular weight from >40,000 to <10,000 were detected.

Among the additional findings of Chang *et al.*, one is of particular

interest, namely that the mtDNA origin of replication is not used at a significant frequency in chimaeric plasmids. In fact, all the *EcoRI* fragments originating from them and having the electron microscopic appearance of replicative intermediates had the length of pSC101; furthermore, their replication origins mapped in positions known to be the pSC101 replication origin.

To sum up this remarkable work, it has been demonstrated for the first time that transcription of eukaryotic DNA in prokaryotes can actually start at signals located on the eukaryotic DNA itself instead of being the result of read through from the plasmid; such transcripts, which appear to be translated in minicells, do not seem, however, to start at natural promoter sites on mtDNA and, therefore, do not correspond to any of the natural transcripts of the mitochondrial genome. This conclusion, which may, perhaps, disappoint the proponents of the prokaryotic origin of mitochondria, suggests that only a mitochondrial RNA polymerase is able to recognise mitochondrial promoters. In this case, the successful expression of eukaryotic genes in prokaryotes may need selective resection of their control elements, which should be replaced by those of plasmid or phage vectors (Murray *et al.*, *Xth FEBS Meeting*, **38**, 193; 1975). It should be recalled, however, that most of the mitochondrial transcripts are, in fact, derived from the processing of the single RNA copy of the H-strand (Aloni and Attardi, *J. molec. Biol.*, **70**, 363, 1972). Such a situation implying the presence of a single promoter on the H-strand, makes animal mtDNA an unfavourable model to test its faithful transcription in minicells and leaves the door open, for the time being, to some optimism over the possibility of correct expression of eukaryotic genes in prokaryotes. An encouraging result along this line is the very recent finding by K. Struhl, J. Cameron and R. Davis of Stanford University of complementation of a *E. coli* *hisB* mutation by a yeast DNA fragment inserted into λ DNA. □

B chromosomes in grasshoppers

from John Bishop

B CHROMOSOMES are chromosomes which appear in some but not in other members of a species, and as such are clearly not necessary for the survival of

individuals. The designation B indicates a supernumerary chromosome, in contrast to the A chromosomes, the representatives of the normal complement.

B chromosomes occur in many species of animals and plants. They are heterochromatic, do not pair with A chromosomes, and have no simple Mendelian effects upon the phenotype. They have been observed, however, to have very sharp clinal distributions which suggests a greater tolerance of B chromosomes under more favourable environmental conditions. The evolutionary significance of B chromosomes is elegantly explored in a paper by Hewitt in *Chromosomes Today* (4, edit. by Wahrman and Lewis, Wiley, New York, 1972).

Several studies have pointed to homologies between different B chromosomes and autosomes, centric fragments and X chromosomes. This suggests the exciting possibility that B chromosomes might represent one of the routes by which the genomes of eukaryotes change radically in informational content to take advantage of changing environmental opportunities. The B chromosome, being heterochromatic and therefore at least largely inactive, would be freed from selective constraint; but could be called on again (perhaps randomly) when and if it fulfilled a newly required function.

Because of this, it was both surprising and disquieting when Gibson and Hewitt (*Nature*, **225**, 67; 1970; *Chromosoma*, **38**, 121; 1972) suggested that the incidence of B chromosomes in the grasshopper *Myrmeleotettix maculatus* was correlated with the occurrence of a DNA satellite which accounted for as much DNA as the B chromosome(s) could be expected to comprise, if not more.

A satellite DNA is one with a different base composition and therefore a distinct buoyant density from the bulk of the DNA (the main-band DNA). Characteristically, a satellite DNA consists of very many similar short sequences (less than 20 nucleotide pairs) arranged tandemly in long continuous stretches (greater than 30,000 nucleotide pairs). On the other hand main-band DNA is largely composed of non-repetitive sequences intimately interspersed with repetitive sequences that have a much lower repetition frequency than satellite DNA.

If B chromosomes contained DNA that had such a degree of disparity with the A chromosome DNA then it would seem unlikely that the two were in any way related. This would very much weigh against the idea that B chromosomes are in the mainstream of the generation of new genetic function. If this were general, it would close, in eukaryotes, an enticing door which has only more recently

IN liquid crystals long molecules are aligned parallel to one another but are disordered and free to move in the plane perpendicular to their long axis. The ends of the molecules may all be in register (smectic) or not (nematic), with all the chains parallel, or in register with successive layers of molecules tilted in different directions (cholesteric). It has always seemed likely that some synthetic polymers might form liquid crystals but until recently there has been no example of this in a pure polymer. Liquid crystal formers have a characteristic melting behaviour. On heating there is a change from crystal to liquid crystal with a large heat of transition followed a few degrees later by a transition with a small heat to the liquid. One should also be able to observe the partial ordering by X-ray diffraction and the molecular motion in the liquid crystalline phase by NMR.

Beatty and co-workers at Xerox (*Macromolecules*, **8**, 547; 1975) have now described an almost perfect example of such melting behaviour in polydiethylsiloxane. Calorimetry and X-ray diffraction provide supporting evidence and restricted molecular motion was observed by NMR and dielectric measurements. In other respects polydiethylsiloxane is a normally behaved semi-crystalline polymer and the amorphous regions of the structure seem unaffected by

the transition in the crystalline parts. Polysiloxane chains are quite flexible although it would seem more reasonable to expect liquid crystalline phases in stiff chains, where the inter-chain bonding could loosen at lower temperatures than those at which the chains could become free to rotate internally.

The morphology of the liquid crystalline phase and the mechanism of the transition from liquid to liquid

Polymeric liquid crystals

from Paul Calvert

crystal and from liquid crystal to crystal in terms of chain folding and spherulite formation have yet to be elucidated. The work of Price and Stein (*J. Phys. Chem.*, **77**, 396, 399, 409; 1973) on the crystallisation kinetics of cholesteric liquid crystals provides a starting point for considering what is expected in the polymeric system.

It is possible that other polymer liquid crystalline phases have been overlooked because of their narrow temperature range. Beatty mentions a series of atactic stiff chain acrylates which have similar X-ray diffraction and melting behaviour to liquid crystals. These materials, however,

do not form true crystals and probably fit better into the grey area between ordered glasses and disordered crystals. A better example is the phase observed to form at high pressures in polyethylene by Bassett (*J. appl. Phys.*, **45**, 4146; 1974). This shows the same behaviour as polydiethylsiloxane though Bassett favours a disordered hexagonal crystal phase at present. He suggests that this phase is the cause of extended chain (large) crystal formation in polyethylene at high pressures. If this is a liquid crystal phase one could expect that extended chain crystals will also grow from polydiethylsiloxane.

There are some examples of solutions of polymers which form liquid crystals. Stiff chain aromatic polyamides in solution in acids form liquid crystals at about 10% polymer. Poly- γ -benzyl-L-glutamate in dichloromethane is similar (Duke and DuPré, *Macromolecules*, **7**, 374; 1974; Hines and Samutski, *Macromolecules*, **6**, 793; 1973), on the face of it, however, these solution systems are a different case from pure polymer.

This would be a good time for polymer physicists to predict the arrangement of chains in the liquid crystalline phase and to predict which other polymers should form liquid crystals. Current theories of polymer crystallisation seem rather *ad hoc* and a successful prediction could add respectability.

been opened towards the prokaryotes (Wallace and Morowitz, *Chromosoma*, **40**, 121; 1973).

The situation reported for *M. maculatus* was not, however, observed in other organisms (see for example Chilton and McCarthy, *Genetics*, **74**, 605; 1973). Furthermore, the *M. maculatus* satellite had most unusual properties: not only did it contain highly-repetitive DNA, as do all DNA satellites, but most of it consisted of DNA sequences which, as far as the satellite was concerned, were unique. This was, so to speak, a unique observation.

From the same group in Norwich, but with different authorship, there came recently a confirmation of the correlation between B chromosome content and the occurrence of satellite in *M. maculatus* (Wilmore and Brown, *Chromosoma*, **51**, 337; 1975). This study pointed up a most interesting coincidence: the buoyant density of the *M. maculatus* satellite is identical to that of *Paramecium aurelia* DNA, to the third decimal place. It also has much the same analytical complexity (compare Gibson and Hewitt, *Chromosoma*, **38**, 121; 1972; and Cummings, *Chromosoma*, **53**, 192; 1975).

Yet more recently, Dover and Henderson (*Nature*, **259**, 57; 1975) have reopened the issue by examining some of the same populations of *M. maculatus* previously examined by Gibson and Hewitt. They did indeed find B chromosomes, but found no satellite, even in the presence of actinomycin D. In fact, grasshoppers as a group seem to be unusually free of satellites (Wilmore and Brown, *op. cit.*, 1975).

The unusual properties of the satellite observed by Gibson and Hewitt offer a conceivable solution to the problem. It shows the characteristics of an entire genome, rather than those common to other eukaryotic satellites, and furthermore, it is uncannily like a ciliate genome. It seems possible, then, that the DNA of some grasshoppers is contaminated by that of a ciliate, perhaps a ciliate parasite. It is not by any means inconceivable that the distribution of such a parasite would correlate, perhaps loosely, with that of B chromosomes.

Whatever the eventual explanation it would seem that B chromosomes may soon return securely to their former place as potential progenitors of genetic diversity in eukaryotes. Perhaps this

is one instance in which a negative finding could ultimately outweigh a positive one. □

The early Earth

from Lynn Margulis

The second College Park Symposium—Chemical Evolution in the Precambrian—was held at the University of Maryland on October 29–November 1. It was sponsored by NASA and the NSF and organised and chaired by Professor Cyril Ponnamperna, Laboratory of Chemical Evolution, Department of Chemistry, University of Maryland. The proceedings will be published by Academic Press, New York, jointly with last year's College Park Symposium on the giant planets.

WHAT were conditions like 4 thousand million years ago? Despite enormous diversity in training, formal back-

ground, methods and scientific approaches amongst the participants agreement on certain basic concepts did emerge at the meeting. V. Rama-Murthy's (University of Minnesota) description of the role of the iron-nickel-lighter substances in the formation of Earth's core and G. Wetherill's (Carnegie Institution of Washington) time scale and planetological-geological framework for the early Precambrian seemed generally accepted. The early planetary period began about 4.43 billion (10^9) years ago (bya) when heavy bombardment of the Earth by Solar System debris occurred and is thought to have gone on until about 3.88 bya. From this time until the present we have at least an intermittent but continuous rock record. A middle period of meteoritic bombardment is recognised between 2.85 and 2.6 bya which corresponds to the principal period of igneous activity on the Moon and is thought to have also been marked by volcanism on the Earth. These events "reset" most of the older geochronological clocks. Challenging previous work by Goldich claiming an age of 3.8 byr, Wetherill and his colleagues claim that the Earth's most ancient rocks reliably aged to date have been found on the coast of Labrador and in Greenland. These old gneisses were probably contiguous some 3.62 bya. Only slightly younger than the oldest rocks themselves is the oldest evidence for life in sediments. J. Henderson (Geological Survey of Canada) described sialic crustal material from the Archaean above a granitic basement about 3.0 byr old (Slave Structural Province, Canada). The oldest presumptive evidence for life here is based on black carbonaceous shales and those laminated heros of the Precambrian stage—stromatolites. Thus, Canada is beginning to compete for attention with the remarkable ancient sediments of the lower Precambrian in southern Africa's Swaziland System.

Whereas the Isua Series (the ancient rocks of southwestern Greenland) of moderately metamorphosed sediments yield a rather dull carbon profile with none of the finely dispersed kerogen-like materials typical of younger rocks, only traces of methane and so forth and rather lunar-like with respect to paucity of carbon, the South African (Swaziland Supergroup) Onverwacht and Fig Tree sediments are much richer. B. Nagy (University of Arizona) and his colleagues found aromatic hydrocarbons (by ozonolysis) and, in the Fig Tree, long chain normal alkanes as well. In the organo-geochemical analyses of the Transvaal stromatolite kerogen considerably more hydrocarbon, oxygen, nitrogen and sulphur compounds are found in the

sediments (2.3 byr). Of course interpretations are risky but it is highly possible that life arose between 3.6 and 3.0 bya and that for a while both chemical and biological evolution continued together. (The possibility that the Fig Tree microspheres are not biological but rather prebiotic chemical fossils was well explored by J. Wm. Schopf (University of California, Los Angeles).)

Although alternative interpretations are possible it seemed that the participants at the meeting would tend to endorse the following scheme. Shortly after the Earth stabilised life evolved and went into Darwinian motion and by 2.7 bya (in Canada and South Africa) bacteria and blue green alga had evolved, diversified, and were flourishing as demonstrated by L. A. Nagy's (University of Arizona) Transvaal microfossils (2.3 byr old) of both coccoid and filamentous forms.

For the history of the atmosphere both J. C. G. Walker (National Radio Observatory, Arecibo, Puerto Rico) and R. Siever (Harvard University) presented arguments that degassing from the interior of the Earth probably occurred during the earliest accretion phase (4.55–4.33 bya) and that most other gaseous emission since then has been recycling rather than accumulating. Everyone seemed to agree with Siever that the earliest Archaean atmosphere was anoxygenic but that in other ways (with respect to weathering, composition and total pressures) the atmospheric-surface processes were not very different from those today. Neither Siever nor Walker (nor anyone else) argued that the Archaean atmosphere containing hydrogen, methane and ammonia was highly reducing. Nor did that seem to bother anyone. For example, C. Folson (University of Hawaii) demonstrated that he could make perfectly good organic microspheres in Urey-Miller type electrical discharges with CO, CO₂ and N₂. In this system, although methane could replace CO₂ as a carbon source, it was not required for the abiotic generation of simple organic "formed bodies". There was agreement with P. E. Cloud Jr's concept that the atmospheric transition from anoxygenic to oxygenic occurred some 2 bya. A common surface mat blue green alga (for example *Lyngbya* sp.) survives potentially lethal quantities of ultraviolet for days when afforded protection by "matting" (inoculum size) and/or sodium nitrate or nitrite in the medium (Rambler, Boston University) so it is likely that even before the appearance of stratospheric ozone, algal mat communities could cope with ultraviolet light.

T. Hoering (Carnegie Institution of Washington) inspired a good deal of

enthusiasm with his preliminary data on hydrogen isotope fractionations found in fossils and presumably generated biologically. Whatever the real cause of these fractionations, deuterium/hydrogen ratios promise to serve us well as a complement to carbon isotope work.

Although everyone agreed that the Precambrian was the "age of the prokaryotes" there was no consensus concerning the time of first appearance of eukaryotic cells. Primary sequence data on primitive proteins such as ferredoxin (D. O. Hall, Kings College, London) and cytochromes (Cohen and M. Dayhoff, Georgetown University) are proving helpful in reconstructing the evolutionary past. Extrapolations from accepted point mutation rates in proteins and RNAs do suggest that Schopf's evidence for the appearance of nucleated algae by the time Bitter Springs deposits were made (850 ± 100 Myr) is very reasonable.

But that rich fossil assemblage may indeed be evidence not for eukaryotes but for the acme of prokaryotic development. Schopf led a lively discussion in which he maintained that "nucleated organisms were extant prior to 850 ± 100 Myr and that the lineage may well have been established as early as $1,400 \pm 100$ Myr ago." Some of our results with artificial "fossils" (using ethyl silicate) and Knoll and Barghoorn's observations on partially degraded blue green algae counsel caution in interpreting the Bitter Springs fossil biota as containing *bona fide* remains of chlorophyte or rhodophyte algae.

The Proterozoic (2 bya) Gunflint stromatolites and microfossils were discussed excitedly in terms of their modern counterparts. *Metallogenium* (a manganese and iron-oxidising microbe discovered by the Russians) bears a striking resemblance to the Gunflint genus *Eoastrian* (E. S. Barghoorn, Harvard University). Everyone welcomed B. Siegel's (University of Hawaii) presentation on newly collected cultures of the enigmatic *Kakabekia barghoorniana*, the microbe that so resembles the Gunflint fossil *Kakabekia umbellata*. Although new data is available on this organism, which has now been collected from diatom-rich cold alkaline places all over the world, the details of its life cycle and culture requirements remain unknown.

Attacking the site of the origin of life itself straight on L. Onsager (University of Florida, Miami) discussed interfacial polymerisation processes that might have occurred at the geochemically derived "primordial oil slick", which, theoretically at least, was conveniently supplied by M. J. Dwyer (University of Philadelphia).

articles

Influence of ancient solar-proton events on the evolution of life

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There is mounting evidence that past extinctions of faunal species have occurred in near coincidence with reversals in polarity of the geomagnetic field. Could the link lie in catastrophic depletions of stratospheric ozone caused by solar-proton irradiation over a reduced geomagnetic field?

WE discuss here a new mechanism that could explain the apparent correlation between faunal extinctions and reversals of the geomagnetic field more satisfactorily than other mechanisms proposed so far.

Uffen has suggested¹ that the surface of the Earth could be exposed to abnormally high fluxes of radiation during the periods of reversal of the polarity of the geomagnetic field (see ref. 2). During these geologically brief periods, the shielding effects of the magnetic field would be removed, or at least severely weakened, allowing galactic cosmic rays to have access to the entire Earth, instead of merely to high-latitude regions, and also causing the radiation belts to precipitate. Uffen proposed¹ that this enhanced level of radiation could lead to increased mutation rates among living species, thereby explaining sudden appearances and disappearances of species from the palaeontological record.

His suggestion has since been examined^{3,4} and it is now generally felt that the effect on living species would probably be insignificant. Nonetheless, there is mounting evidence that a correlation does exist between major faunal extinction and geomagnetic polarity reversals. The validity of this correlation in fairly recent geological time seems to have been well established by studies of fossil species of radiolaria (single-celled, marine microorganisms). Using deep-sea cores obtained from a variety of locations, Hays⁵ has determined that during the past 2.5 Myr eight species became extinct, and that six of those disappeared in near coincidence with polarity reversals.

Hays has estimated that the probability of a chance coincidence between faunal extinctions and polarity reversals is less than 0.002 (see also refs 6 and 7). Going back still further in time, there is more tenuous evidence of a general correlation between polarity reversals and extinctions of both marine and terrestrial fauna^{8,9}. The establishment of a clear case for the radiolarian extinctions has only been possible through a fortunate combination of circumstances that allows the distinctive fossilised siliceous skeletons of the radiolaria to be studied in direct relationship to the geomagnetic record in the same deep-sea cores.

Hays' work^{5,7} has shown that individual species can survive through several polarity reversals, though they may often

decrease in numbers at the time of a reversal, before finally disappearing. This suggests that the harmful effects accompanying a polarity reversal, whatever they may be, form only one component of the total environmental stress on a given species. Extinction may result from the addition of this factor to others that are already subjecting the species to severe stress at the time of the reversal.

Other mechanisms linking changes in the geomagnetic field with effects on living organisms have been proposed, include climate changes¹⁰ and direct magnetic effects on growth^{5,9}, among others. None has yet received acceptance, however.

Many solar flares generate fluxes of energetic particles, mostly protons, at the Earth. These events occur sporadically, though their frequency of occurrence is strongly dependent on the general level of solar activity manifested in the 11-yr solar cycle. The energies of the individual particles cover a very wide range, from roughly 10⁴ eV to over 10⁹ eV, and the intensities and spectra are extremely variable from event to event. Though the fluxes of particles capable of reaching the lower atmosphere or ground level (that is, those with energies in excess of 10⁹ eV) are generally small and of short duration, however, very substantial fluxes, often persisting for many days, reach the stratosphere during many events.

Crutzen *et al.*¹¹ have shown that the ionisation produced in the stratosphere by these particles leads to the formation of nitric oxide in large quantities through the dissociation and the dissociative ionisation of N₂, followed by the reaction of N atoms with oxygen. A few intense events a year can produce NO in amounts comparable with those produced by the main known source (the oxidation of N₂O), and considerably larger than those produced by galactic cosmic rays. Ozone is catalytically destroyed by NO (ref. 12) chiefly through the pair of reactions



thus directly affecting the efficiency of the ozone shield that protects the surface of the Earth from potentially harmful solar ultraviolet radiation. Although each solar-proton event lasts only a few days, the lifetime of NO in the stratosphere is long, and its effect on the ozone layer is likely to persist for several years.

At present, the geomagnetic field effectively guides the solar protons towards high geomagnetic latitudes, and for most practical purposes the direct effects on the atmosphere are confined to geomagnetic latitudes higher than about 60°. The long lifetime of NO ensures that it will be spread over the Earth by horizontal transport, but it will be diluted by a geometrical

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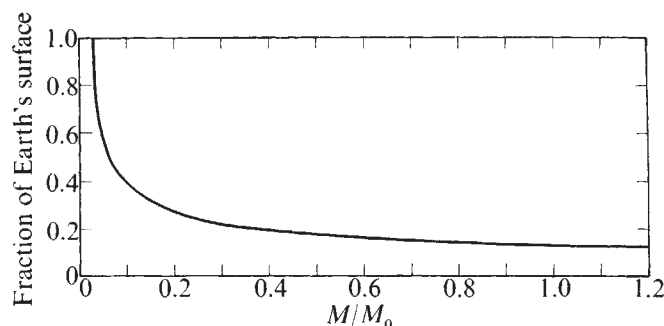
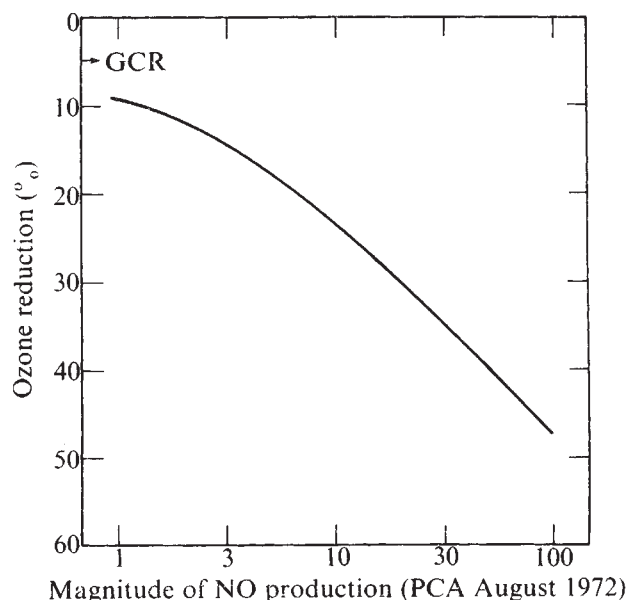


Fig. 1 An approximate estimate of the fraction of the global stratospheric surface exposed to solar protons of 30 MeV energy as the Earth's dipole moment, M , is changed from its present value M_0 .

factor of about 7, corresponding to the fraction of the Earth lying at latitudes higher than 60° . During polarity reversals, however, the dipole component of the geomagnetic field probably weakens or disappears for periods of a few thousand years¹³, allowing both solar protons and galactic cosmic rays access to much larger areas of the Earth, and leading to much more severe effects on the ozone shield than are possible at present. Biological species that had evolved over the preceding several million years of geomagnetic stability may be unable to survive the harsher ultraviolet environment, and would presumably be replaced by other species with more adaptability. It is worth noting that many contemporary simple forms of aquatic life seem to be intolerant of enhanced ultraviolet radiation in the 300-nm wavelength range¹⁴, which can penetrate water to depths of several metres¹⁵.

This mechanism is related to one proposed by Ruderman¹⁶, who suggested that the sporadic occurrence of supernovae in the past might have subjected the Earth to intense fluxes of radiation, resulting in a removal of the ozone shield through the catalytic action of NO. This mechanism should certainly be considered, but it cannot explain the observed correlation between major faunal extinctions and polarity reversals. The solar-proton hypothesis, on the other hand, provides at least a tentative explanation of this mysterious relationship.

Fig. 2 Fractional depletion of the total content of the ozone column of the stratosphere produced by intense solar-proton events in the absence of a geomagnetic field. The events are assumed to have the same spectrum as that of the August 1972 event, and the abscissa indicates the particle flux in units of the August 1972 flux. GCR, the calculated depletion produced by the continuous action of galactic cosmic rays.



Besides undergoing polarity reversals, the intensity of the geomagnetic field varies on a time scale of thousands of years. There is no evidence for any biological effects related to this variation, and a possible explanation is illustrated in Fig. 1. This shows the fraction of the global atmospheric surface accessible to protons of 30 MeV energy as a function of the geomagnetic dipole moment, normalised to unity at its present value. Since 30 MeV is roughly the minimum energy needed to reach the stratosphere, this quantity represents the fraction of the stratosphere that is exposed to the full available solar-proton flux. The calculation is approximate, based on a simple dipole field, in which the Størmer theory of geomagnetic cutoffs is applicable, with a 5° correction applied to take account of the distortion of the outer regions of the field by the solar wind¹⁷. Figure 1 shows that the dipole moment must be reduced by a factor of 10 or more before the area directly affected by solar protons is greatly increased. Evidence from rock magnetisation studies indicate that the field intensity does decrease by an order of magnitude during polarity reversals, but that it does not completely disappear. The remaining field is likely to comprise

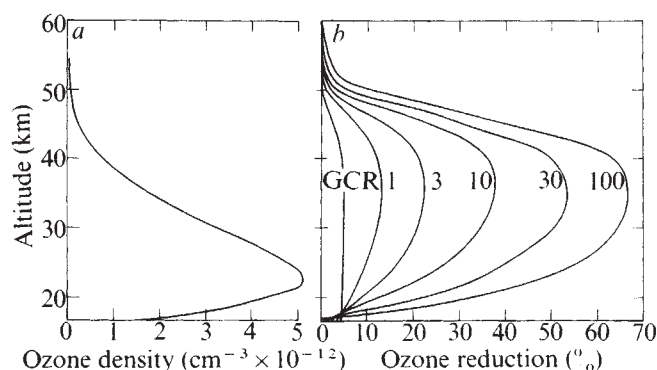


Fig. 3 *a*, Calculated ozone height profile for normal conditions; *b*, fractional depletion produced by solar-proton events of the indicated magnitudes (relative to the event of August 1972), and by galactic cosmic rays (GCR).

mainly non-dipole components whose effect on energetic particles is impossible to estimate. It is likely, however, that non-dipole fields will allow freer access than would dipole fields of similar strength. We assume here that the field actually disappears during polarity reversals.

Solar-proton events have been studied only during the past 15–20 yr. Based on the accumulated evidence, it is difficult to estimate the maximum intensity that an event may reach during an episode of polarity reversal lasting perhaps a thousand years or more. The most intense event known was that of August 1972, during which about 2.5×10^{15} erg cm^{-2} entered the high-latitude atmosphere in the form of protons with energies greater than 30 MeV, which penetrate into the stratosphere. It does not seem that events one or two orders of magnitude more intense than that of August 1972 can be ruled out when the observation period is lengthened to more than 1,000 yr. The quantity of NO that can be produced during extremely intense events is not unlimited, however, since the reaction



which re-creates N_2 from dissociated N atoms, assumes major importance when the NO concentration is high.

Figure 2 shows our estimate of ozone reductions resulting from the increased stratospheric production of NO by the continuous action of galactic cosmic rays and by sporadic solar-proton events occurring during polarity reversals. It is assumed that during these periods the atmosphere is otherwise unchanged from its present state. The calculations were carried out using a one-dimensional, time-dependent, photochemical model, which is an extension of a model developed by one of us (P.J.C.), and which will be described elsewhere. The production rates

of NO from galactic cosmic rays were obtained from a study by Brasseur and Nicolet¹⁸. The magnitudes of the solar-proton events are here measured in terms of the August 1972 event, but it must be remembered that in reality different events differ greatly in their particle energy spectra as well as their fluxes. The numbers probably represent underestimates of the effects, since the conditions of the model calculations were chosen to simulate the effects of the solar protons at low latitudes. As NO production from the oxidation of N₂O is a maximum at low latitudes, the contribution of NO from solar-proton events relative to that from N₂O oxidation is larger on a global scale than is indicated by the present model.

The calculations show that very substantial ozone reductions must accompany intense solar-proton events during polarity reversals, and also that the maximum effect is reached 1–2 yr after the event, and the time required for the atmosphere to recover to its initial condition is almost 10 yr. Even in the absence of intense solar-proton events, the ozone content will be reduced by 4.3% by the continuous action of galactic cosmic rays.

The altitude dependence of the ozone reduction is shown in Fig. 3, which indicates that the main effect occurs well above the maximum of the ozone layer, at least near the time of peak reduction. Figure 4 shows the percentage change in the solar heating rate attributable to the absorption of ultraviolet and visible radiation by O₃ and NO₂. It may, incidentally, be concluded that changes in the Earth's climate may result from intense solar-proton events occurring during polarity reversals. In fact, during very intense events enough NO₂ could be formed in the stratosphere to affect significantly the penetration to ground level of visible radiation in the 400–500-nm range, where NO₂ absorbs radiation rather strongly.

We have shown that intense solar-proton events occurring during polarity reversals may cause large reductions in the atmospheric column content of ozone. The effect of such depletions on living organisms is difficult to estimate, but Fig. 5 gives a rough indication of the magnitude of the effects. Assuming a thickness of the ozone layer of 0.3 cm, and adopting the solar fluxes of Ackerman¹⁹, the total number of photons incident per cm² during an equinoctial day at the Equator has been calculated as a function of wavelength in the 270–320-nm range. Fluxes at each wavelength have been multiplied by a 'relative biological effectiveness' factor²⁰, based on the response of protein and nucleic acids to ultraviolet radiation. The calculations show that during a polarity reversal a solar-proton event of the same magnitude as the August 1972 event would increase the effective dose by 15%, and that events 10 and 100 times more intense would increase the dose by 55% and 160%, respectively. Such increases would inevitably have some effect on simple freshwater

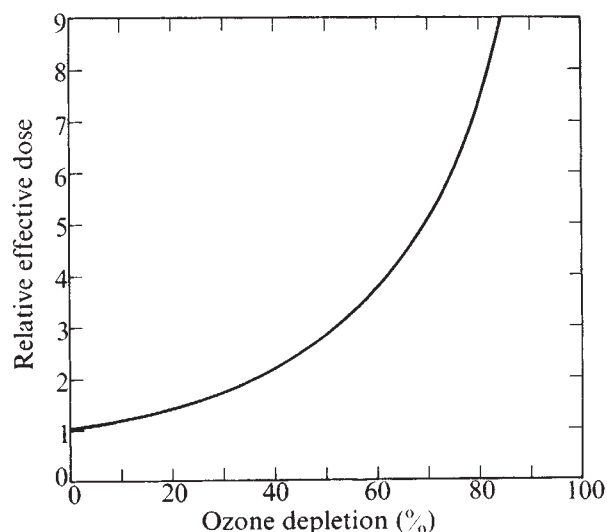


Fig. 5 The relative effective dose of ultraviolet radiation received on an equinoctial day at the Equator as a function of the depletion in stratospheric ozone.

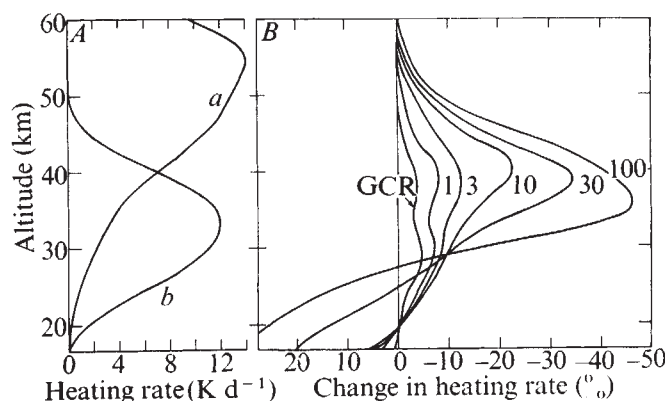
microorganisms, many of which at present seem to be living close to their maximum tolerance of ultraviolet radiation¹⁴. The effect on marine organisms is unknown, and will depend on such factors as the length of time spent in the upper part of the euphotic zone, where the ultraviolet dosage will be highest, and on the effect of the increased ultraviolet light on phytoplankton. The total effect on the organisms, however, is certainly an increase in environmental stress, which may, as already mentioned, be sufficient to lead to extinction.

Though the radiolarian extinctions discussed here may have resulted from the addition of one new stress factor to others that existed already, it is perhaps worth speculating that the mechanism we have described may have had the dominant role in some of the massive faunal extinctions that have occurred in the distant past. For example, roughly one-third of all living species became extinct at the close of the Cretaceous, which was a period marked by a resumption of polarity reversals, following a very lengthy period of normal polarity²¹.

In conclusion, it seems that current concern about possible anthropogenic destruction of stratospheric ozone may be well founded, since it is possible that major ozone depletions occurring in the distant past have had a profound effect on the development of life as we know it.

We acknowledge a discussion with F. E. M. Lilley which led to the initiation of this study. The National Center for Atmospheric Research, Boulder, is supported by the National Science Foundation.

Fig. 4 A, Calculated heating rate under normal conditions attributable to the absorption of solar radiation by: a, O₃; and b, NO₂ ($\times 10^3$). B, Fractional changes attributable to intense solar-proton events and galactic cosmic rays during polarity reversals. The increased heating rates at the lower levels are attributable to the increased penetration of solar ultraviolet radiation when the overlying ozone is depleted.



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Thickening of the Ross Ice Shelf and equilibrium state of the West Antarctic ice sheet

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Data from the south-east quadrant of the Ross Ice Shelf, Antarctica indicate that, near the grounding line, the ice shelf is growing thicker by almost 1 m yr^{-1} . This thickening rate implies an advance of 1 km yr^{-1} of the grounding line between the West Antarctic ice sheet and the Ross Ice Shelf. It can be reconciled with independent evidence for current thinning of the West Antarctic ice sheet if the latter is a delayed response to an earlier retreat of the ice shelf grounding line. The high rates of ice shelf thickening may be due to an increase in the resistance to ice shelf movement caused by localised ice shelf grounding following isostatic uplift of the sea bed.

Most of the West Antarctic ice sheet (Fig. 1) rests on rock that is below sea level now and would still be below sea level if the ice sheet were removed and complete isostatic rebound occurred. Mercer¹ has suggested that such an ice sheet could disintegrate rather rapidly if the surrounding ice shelves were removed, and Hughes² noted that the surface profile of the West Antarctic ice sheet (WIS) was not an equilibrium profile and suggested that the ice sheet may currently be disintegrating. Here I shall use measurements made by the Ross Ice Shelf Project (RISP) (refs 3, 4 and H. B. Clausen and W. Dansgaard, personal communication) to examine the state of equilibrium of parts of the Ross Ice Shelf (RIS). I shall correlate this information with other available data to evaluate current behaviour of the WIS.

Data

The results from RISP are summarised in Fig. 2 where strain rates, accumulation rates, and ice velocities are plotted against distance along the flow line that passes through the site of a proposed bore hole through the ice shelf and into the sea bed. The path of the flow line across the ice shelf was deduced from measurements of ice velocity and surface strain rate³. It enters the ice shelf from ice stream 'B' (Fig. 1) and has been extrapolated on to the WIS assuming the ice to be moving perpendicular to the surface contours. The catchment area for ice stream 'B' is also shown in Fig. 1. Snow accumulation rates (A) in the catchment area (Fig. 2) are from Bull⁵, and local ice velocities (V) have been calculated assuming that ice movement exactly compensates upstream snow accumulation. Vertical strain rates (ϵ) in this area were then calculated from the equation of continuity⁶

$$\partial H / \partial t + V \partial H / \partial x = A + B + H \epsilon \quad (1)$$

where x is distance along the flow line, H is ice thickness, $\partial H / \partial t$ is the rate of thickening of the ice sheet, $\partial H / \partial x$ is the thickness gradient along the flow line, B is bottom freezing rate (A and B are expressed as depths of ice per unit time; on the WIS I have assumed that $|B| \ll |A|$) and ϵ is negative for thinning. To calculate values of ϵ upstream of the grounding line, I have assumed that in this area $\partial H / \partial t = 0$. In the central part of the ice shelf A and V values have been interpolated

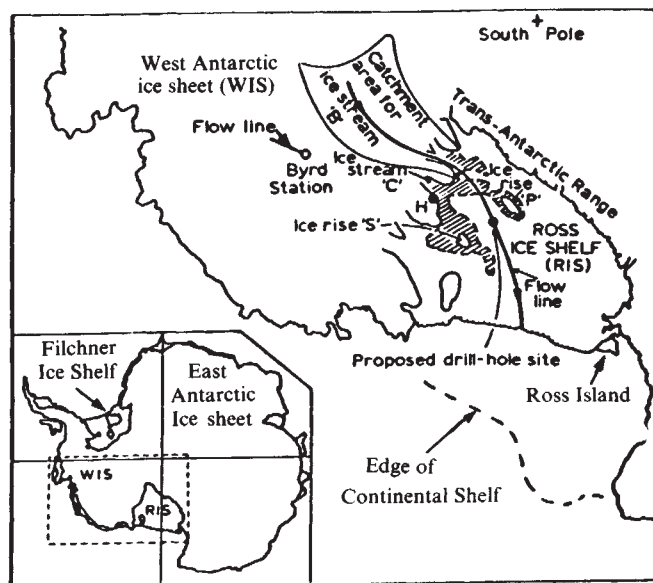
between the RIPS measurements and data from near the ice front^{7,8}, and for this region I have assumed zero lateral strain, so that $\epsilon = -\partial V / \partial x$. This assumption is probably valid near the ice front⁷, but further upstream there may be lateral compression because of converging ice streams. Ice thickness data^{4,8-10} are most reliable for the ice shelf section of the flow line.

Interpretation

The data shown in Fig. 2 has been used to calculate steady-state snow particle paths¹¹ from where they land on the surface to the point of exit from the ice shelf (Fig. 3). Ice velocity is assumed to be independent of depth, which is true for the ice shelf and is a good approximation for most of WIS, where ice movement is predominantly by bottom sliding¹². The particle-path results give the relationship between age and depth at all points along the flow line, and Fig. 4 is a plot of age against depth at the site of the proposed drill hole, but inevitably, these ages can be regarded as only approximate. Furthermore, the steady-state assumption (that conditions along the flow line are invariant with time) is a rather drastic restriction. Indeed 10,000 yr ago the WIS probably covered the entire Ross Ice Shelf. Strain rates and ice velocities would then have been considerably smaller than those in Fig. 2. Lower strain rates imply a slower thinning of the annual layers and, even if snow accumulation was lower over a more extensive WIS, the deepest ice is probably younger than shown in Fig. 4.

Where the particle paths leave the base of the ice shelf, bottom melting must occur for the ice shelf profile to be time

Fig. 1 Part of the West Antarctic ice sheet and the Ross Ice Shelf. In the shaded area, the sea bed is within 50 m of the ice shelf bottom; this entire region may be grounded within the next 200 yr.



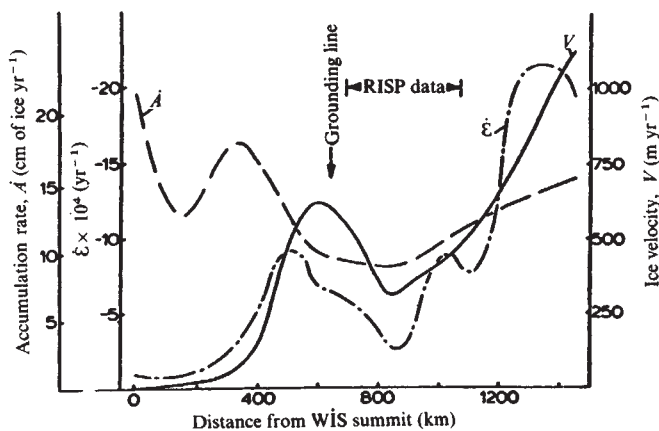


Fig. 2 Snow accumulation rate ($\dot{A}$), ice velocity (V) and vertical strain rates ($\dot{\epsilon}$) plotted against distance along the flow line that passes through the proposed RISP drill-hole site.

invariant. Similarly, bottom freezing occurs where particles enter the base of the ice shelf. Figure 3 indicates that, for a steady state, appreciable bottom melting is taking place near the junction, or grounding line between the WIS and the RIS, with the bottom freezing nearer the ice front. We should, however, expect the opposite to be true with maximum bottom freezing near the grounding line. Indeed, observations of sediment laden ice near ice rise 'P' (ref. 13), where severe fracturing has probably overturned parts of the ice shelf, suggest that bottom freezing is taking place upstream of the ice rise. Consequently, we relax the steady-state assumption, and use equation (1) to study the non-steady-state behaviour of the ice shelf.

Non-equilibrium behaviour

Equation (1) has been solved to give $(\partial H/\partial t - \dot{B})$ for the ice shelf section of the flow line, and the results are given in Fig. 5. Upstream of the drill-hole site $\dot{B}$ probably has a small positive value, but for simplicity we shall assume that $\dot{B} = 0$. Figure 5 then becomes a plot of the rate of ice shelf thickening $(\partial H/\partial t)$ against distance from the grounding line and, within the limits of observation error, $\partial H/\partial t$ decreases linearly from $\sim +1 \text{ m yr}^{-1}$ at the grounding line to zero near the drill-hole site. An interesting effect of this linear decrease in $\partial H/\partial t$ is that the magnitude of the thickness slope $|\partial H/\partial x|$ (and thus, from equation (1), of $\partial H/\partial t$) increases with time. Downstream from the drill-hole site the ice shelf appears to be in equilibrium with bottom melting within $\sim 200 \text{ km}$ of the ice front, as is approximately depicted by the dashed curve. The large deviations from the dashed curve almost certainly arise from over-estimation of the relevant vertical strain rates, which were calculated assuming lateral strain to be zero. In reality there is probably lateral compression from the converging ice streams.

It is important to note that the high rates of ice shelf thickening shown in Fig. 5 near the grounding line were calculated solely from the RISP data and have an accuracy of $\sim \pm 0.2 \text{ m yr}^{-1}$. Although this is the only region where we have 'hard' data, the results shown in Fig. 5 are of particular interest since they imply an annual advance of the grounding line in this area of $\sim 1 \text{ km}$, and if the ice shelf continues to thicken at its present rate it will run aground on the bed rock, 100 km upstream of the drill-hole site (Fig. 3), in $\sim 50 \text{ yr}$. It should be stressed that such rapid thickening is not expected to be typical for the entire grounding line; indeed data from an area where the ice is moving by slow 'sheet flow' (H in Fig. 1) indicate that the local grounding line is almost stationary. Nevertheless, ice streams are responsible for most of the WIS drainage, and their present behaviour is a good indicator for the probable future behaviour of WIS.

Interpretation

The high rates of ice shelf thickening near the grounding line are of considerable interest, particularly since other evidence¹⁴⁻¹⁷ suggests that the WIS has thinned appreciably during the past 10,000 yr and may still be thinning. Equation (1) can be solved using measurements from Byrd Station (Fig. 1)^{2,12} to give $(\partial H/\partial t - \dot{B}) \approx -0.4 \text{ m yr}^{-1}$. $\dot{B}$ is probably $< 10^{-2} \text{ m yr}^{-1}$ (ref. 12), so the ice at Byrd Station is currently thinning by $\sim 0.4 \text{ m yr}^{-1}$. Upstream from Byrd Station, the available data^{2,12} indicate that the vertical strain rate drops, to give a thinning rate of $\sim 0.1 \text{ m yr}^{-1}$ near the WIS summit.

Analysis of the concentration of microparticles in ice from a bore hole at Byrd Station gives estimates of age as a function of depth¹⁸. These can be used to derive historical vertical strain rates, and the record for the past 2,000 yr shows good agreement with the recently measured values. For $\sim 25,000 \text{ yr}$ before 2,000 yr BP, however, vertical strain rates were between 5% and 10% of the present values upstream from Byrd Station. Probable temporal variations in snow accumulation cannot account for this large apparent reduction in strain rates before 2,000 yr BP. Instead, I suggest that a real increase in strain rates took place $\sim 2,000 \text{ yr}$ ago. If we assume that present-day vertical strain rates ($\dot{\epsilon} \approx -10^{-4} \text{ yr}^{-1}$) upstream of Byrd Station are typical for the past 2,000 yr we can estimate the 2,000 yr BP ice thickness as

$$H = H'/(1 + \dot{\epsilon})^{2,000} \quad (2)$$

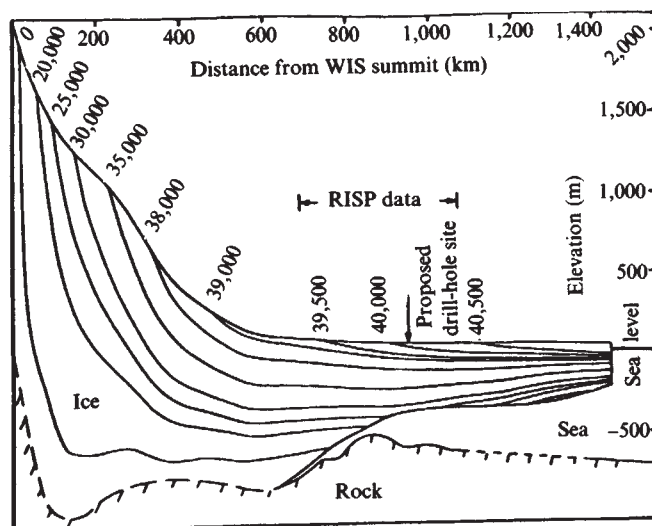
where H' ($\sim 2,500 \text{ m}$) is the present average height above bed rock of the 2,000 yr BP ice surface, giving $H \sim 3,050 \text{ m}$, or $\sim 350 \text{ m}$ thicker than the present ice sheet. This is in agreement with general conclusions from stable isotope analysis of ice from the Byrd Station bore hole¹⁴ and from interpretation of temperatures measured in the bore hole¹⁵.

Near the summit of an ice sheet $V\partial H/\partial x \rightarrow 0$ and probably $\dot{B} \ll \dot{A}$ so equation (1) can be written:

$$\partial H/\partial t \sim \dot{A} + H\dot{\epsilon} \quad (3)$$

With the pre-2,000 yr BP vertical strain rate ($\sim -10^{-5} \text{ yr}^{-1}$) equation (3) gives $\partial H/\partial t \sim \dot{A} \rightarrow (\dot{A} - 0.03) \text{ m yr}^{-1}$, for $H = 0 \rightarrow 3,000 \text{ m}$. The average value now of $\dot{A}$ upstream of Byrd Station is 0.17 m yr^{-1} (ref. 19), and even assuming a significant

Fig. 3 Steady-state particle paths for the flow line that passes through the proposed RISP drill-hole site. The numbers at the point of origin of each flow line represent the time taken in years to pass each point for a snow particle deposited 20 km downstream from the WIS summit. Sea depth was measured in the RISP area⁴ and near the ice front⁸.



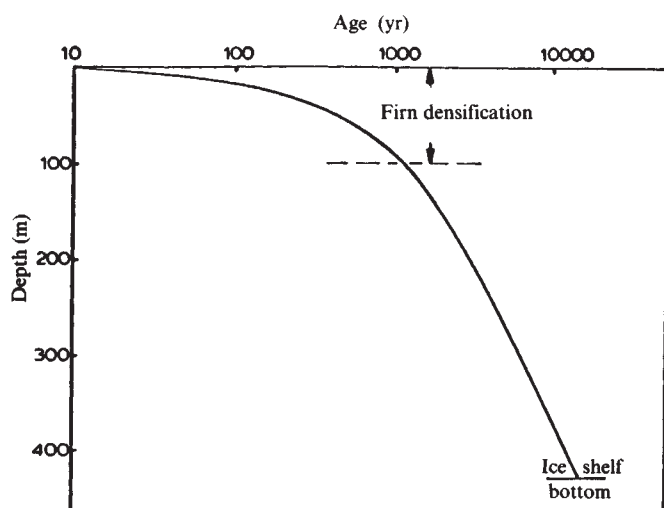


Fig. 4 A plot of age as a function of depth at the proposed RISP drill-hole site based on steady-state assumptions. Actual ice sheet behaviour implies that deeper ice is probably younger than shown.

reduction in A associated with more widespread glaciation we may conclude that the WIS summit was thickening continuously until $\sim 2,000$ yr BP, when thinning commenced. This implies that the WIS failed to achieve an equilibrium state, and is compatible with the theory²⁰ that the WIS can exist only in a state of growth or shrinkage unless its outer margins reach to either a point where the sea has a critical depth or the edge of the continental shelf. It is important to note that the quantitative estimates of ice thickness and rates of thickening before 2,000 yr BP are very approximate, and improvement in their accuracy must await data from drill holes upstream from Byrd Station. It is, however, probably safe to conclude that the ice thickness at the WIS summit 2,000 yr BP was significantly greater than today, and that before 2,000 yr BP the ice sheet had been thickening for $\sim 25,000$ yr. Moreover, analysis of microparticle concentrations in the Byrd Station bore hole indicates that the basal ice is $\sim 27,000$ yr old, implying that the WIS grew on a virtually ice-free area to achieve a summit thickness of 3,000 m over a period of $\sim 25,000$ yr. This time period is in fair agreement with theoretical estimates of ice sheet growth rate²¹. Independent evidence^{16, 22} suggests that the WIS extended almost to the edge of the continental shelf until $\sim 10,000$ yr BP, when recession commenced, and ungrounding had occurred in the Ross Island area (Fig. 1) by 6,000 yr BP. Theory (R. H. Thomas, in preparation) indicates that the retreat of the WIS grounding line would proceed rather slowly until it reached Ross Island, when retreat rates would increase to > 1 km yr⁻¹. Thus WIS recession could have ceased by $\sim 4,500$ yr BP, implying that the thinning of the ice sheet summit now observed is a delayed response to the retreat of the grounding line.

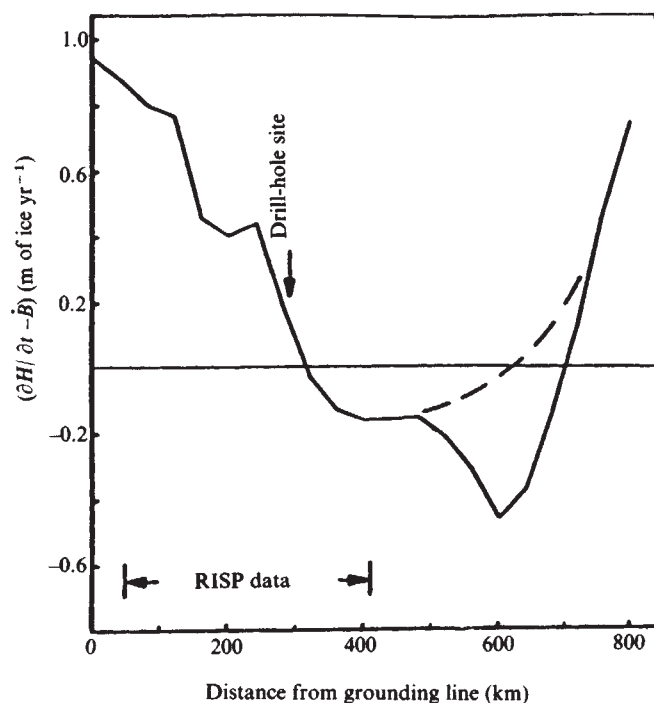
Connection with RISP data

We must now reconcile these conclusions with the RISP observations that imply thickening at the point where ice stream 'B' enters the ice shelf. This thickening must have commenced after the period of major recession, as a consequence of either increased outflow or increased upstream constraint acting on the ice shelf near the grounding line. Increased outflow could result from upstream thinning or from enlargement of the catchment area to include that of ice stream 'C', which currently seems to be relatively inactive. We might, however, expect that present-day outflow rates are appreciably less than those during the stage of maximum recession when the ice shelf was thinning. So we shall examine the second alternative, that upstream constraints on the ice shelf increased during the past 4,500 yr

causing a decrease in the magnitude of ice shelf strain rates and, from equation (1), a positive thickening rate.

At present, the ice shelf in this area is constrained by ice rise 'P' (Fig. 1), an area where the ice shelf has grounded. Removal of the ice rise would allow more rapid ice shelf thinning and probably a grounding line recession in the south-east corner of the RIS. Thus, we can explain the present ice shelf thickening if we assume that grounding of ice rise 'P' took place comparatively recently, after isostatic uplift of the sea bed. An ice sheet completely covering the RIS would have depressed the sea bed in the area of ice rise 'P' by several hundred metres and, for a sufficiently rapid recession, much of this depression could persist until most of the present RIS had formed. Thereafter, isostatically uprising sea-bed highs could ground the ice shelf, thereby restricting continued recession of the WIS, and possibly initiating an advance of the grounding line. Evidence to support this suggestion is provided by the dimensions of ice rise 'P'. An approximate estimate of the relationship between the surface elevation and area appropriate to an ice rise can be expressed²³ in terms of the snow accumulation rate, the ice flow-law parameters and temperature-depth curve for the ice rise. Using current accumulation rates (H. B. Clausen and W. Dansgaard, in preparation), theoretical temperatures²⁴ and ice shelf flow-law parameters²⁵, the equilibrium summit elevation for ice rise 'P' is found to be ~ 200 m greater than observed, indicating that the ice rise is probably growing. Moreover, solution of equation (1) using data from the partially grounded ice shelf upstream of ice rise 'S' (Fig. 1) gives thickening rates up to 0.5 m yr⁻¹, if we assume no bottom freezing. The ice thickness in this area is approximately equal to that of nearby freely floating ice shelf, and it seems likely that local grounding has only recently taken place. The shaded area of ice shelf in Fig. 1 indicates where the sea bed is within 50 m of the bottom of the ice shelf. If the ice shelf continues to thicken at the rates calculated above, then the WIS will advance to cover most of this

Fig. 5 A plot of $(\partial H/\partial t - B)$ against distance from the ice shelf grounding line for the flow line that passes through the proposed RISP drill-hole site. $\partial H/\partial t$ is the thickening rate for the ice shelf, and B is the bottom-freezing rate. Observation errors in the RISP data limit accuracy of corresponding values of $(\partial H/\partial t - B)$ to ± 0.2 m yr⁻¹. Near the grounding line B probably has a small positive value and the plot indicates a rather high rate of ice shelf thickening in this area.



area within the next 200 yr. Further advance will then be delayed by the probable lack of grounded areas in the western part of the ice shelf.

Isostatic uprise rates were at a maximum immediately following removal of the ice load, and the grounding of ice rise 'P' probably occurred shortly after the WIS recession, or ~ 4,000 yr ago. Before grounding, the local ice shelf was probably ~ 500 m thick, with its base ~ 450 m below sea level. The present bed rock high beneath the ice rise 'P' is ~ 250 m below sea level, giving an uplift of 200 m during a period of ~ 4,000 yr. Thus, the average uplift rate is ~ 0.05 m yr⁻¹ compared to a current uplift rate of 0.01 m yr⁻¹ in Fennoscandia, where the remaining uplift²⁶ is of a similar magnitude to that estimated²⁷ for the sea bed beneath the RIS. Estimates of remanent uplift are, however, subject to rather large errors, and the RIS area may be appreciably further from equilibrium than Fennoscandia. Moreover, differences in crustal thickness could result in larger uplift rates beneath the RIS.

Conclusions

The WIS recession that began ~ 10,000 yr ago was probably initiated by the rise in sea level associated with melting of the northern ice sheets, but we should also consider the possibility of isostatic depression of the sea bed triggering recession of the grounding line. Theory²⁰ predicts that an ice sheet grounded below sea level exists in equilibrium when outflow because of snow accumulation on the ice sheet is balanced by movement of ice across the grounding line into a surrounding ice shelf. This occurs at a critical sea depth D , which is a function of ice sheet diameter, snow accumulation rate and the flow properties of ice. The ice sheet grows if sea depth is $< D$, and it recedes if sea depth is $> D$. For a growing ice sheet, isostatic depression of the bed rock continues after the grounding line has reached the appropriate critical sea depth. Moreover, crustal rigidity would cause downwarping of the sea bed for an appreciable distance to seaward of the grounding line. Consequently, sea depth at the grounding line may ultimately exceed D and trigger ice sheet recession.

If growth and decay of WIS is controlled by isostatic movements of the sea bed then we may tentatively conclude: first, that the growth rate and maximum extent of the ice sheet are determined by snow accumulation rates and sea bottom topography. Second, that the longevity of the ice sheet is determined by the rate of isostatic depression of the sea bed near the

ice sheet-ice shelf junction. Third, that after a slow start, recession proceeds very rapidly until the ice shelf formed around the shrinking ice sheet is grounded by isostatically uprising sea bed highs. Thereafter, recession is slowed and may give way to a period of ice sheet growth. Thus the minimum size of the WIS is determined by the availability of suitable obstructing ice rises and, on a sufficiently flat sea bed, almost complete disintegration of the WIS is possible. The bottom topography is, however, altered by erosion and deposition during ice sheet growth and decay so that each cycle of growth and recession follows an individual pattern that is determined by the prevailing sea bed topography. Fourth, that climatic changes probably have only a minor effect on the growth-decay sequence of the WIS. The converse may, however, not be true. In particular, complete disintegration of the WIS may alter world climate sufficiently to initiate a glacial epoch^{28,29}.

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Amino acids in modern and fossil woods

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Amino acids have been found in fossil woods. The extent of amino acid racemisation increases with the age of the wood. The degree of racemisation of proline and hydroxyproline in wood from Kalambo Falls, Zambia, was used to estimate an age of >110,000 yr for the Acheulian-Sangoan transition.

WOOD is found preserved in many geological formations. Wooden tools and artefacts have also been found at many

archaeological sites. The radiocarbon dating method was first applied to a wooden artefact, an acacia wood beam recovered from the tomb of King Zoser in Sakkara, Egypt¹. Radiocarbon dating however cannot be used for objects $\geq 40,000$ yr old. A wood-dating technique capable of extension beyond this present limit would be extremely useful to both anthropologists and geologists, and amino acid racemisation may provide such a technique.

The dating of fossil materials using the racemisation of amino acids is becoming well established. The kinetics and mechanism of the amino acid racemisation reaction and the use, problems and criticisms of this reaction in dating fossil materials have been discussed elsewhere^{2,3}. The method was applied to deep-sea sediments, and fossil bones and shells^{2,3}.

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Also, racemisation in tooth enamel has been used to assess the ages of living mammals⁴. These applications all involve racemisation in inorganic matrices such as calcium carbonate and calcium phosphate, but racemisation has also been shown to occur in an organic matrix, coprolites⁵.

Wood has an organic matrix composed mainly of cellulose, but with a complex biochemical composition because of the numerous biological processes occurring within the tree^{6,7}. Modern woods are known to contain amino acids⁸⁻¹¹, but there have been no investigations of the concentrations and enantiomeric composition of amino acids in fossil woods. We report here the amino acid composition and extent of racemisation in several modern and fossil woods, and a possible interpretation of these results. We emphasise that, because of the complexities and variabilities of plant biochemistry and the paucity of data on modern and fossil woods, this interpretation is preliminary.

Method

The modern and fossil woods we have investigated are listed in Table 1. The samples were first cleaned by ultrasonication in doubly-distilled water to remove surface dirt, and were then carefully broken up into small pieces. After being rinsed in 2 N HCl, the pieces were hydrolysed in excess doubly-distilled 6 N HCl for 24 h. The hydrolysate was centrifuged to remove suspended materials, evaporated to dryness, and the residue desalted on Dowex 50W-X8 (100-200 mesh). Part of the sample was analysed on the Beckman-Spinco Model 118 Automatic Amino Acid Analyser to determine the total amino acid composition. Another fraction of the sample was analysed for enantiomeric ratios either by preparing diastereomeric dipeptides using a modification¹³ of the method of Manning and Moore¹², or by gas chromatography of the N-trifluoroacetyl (+)-2-butyl esters¹⁴.

Total amino acid concentration

The total amino acid analyses (excluding basic amino acids) are shown in Table 1. Several interesting features are apparent. First, the total amino acid concentration per g of dry wood decreases with the age of the sample. This may arise from the hydrolysis of the bound amino acids to free amino acids (or decomposition to other organic compounds). Free amino acids, being more mobile, are later removed from the wood. A similar mechanism accounts for the loss of amino acids in fossil bones¹⁶. To demonstrate that the amino acids in fossil

woods are in the bound rather than in the free state, cleaned, sonicated wood slivers of bristlecone pine were stirred at room temperature for 60 h in 6 N HCl. After this treatment, the HCl solution was evaporated, and analysis of the remaining residue indicated the presence of few, if any, amino acids. Acid hydrolysis of the same slivers, however, yielded amino acids.

Amino acid ratios

Another trend shown in Table 1 is the change in the relative ratios of the amino acids with time. With increasing age, the amounts of aspartic acid, threonine, and serine decrease relative to valine, while the relative amounts of isoleucine, leucine, and glutamic acid remain approximately constant. Proline is one of the most stable amino acids, and becomes predominant in the older woods. In both Kalambo Falls samples, proline constitutes ~25% of the total amino acid present. Kinetic studies carried out by heating samples of bristlecone pine to high temperatures, predict an amino acid stability pattern similar to that observed in the fossil woods¹⁷. This suggests that the amino acids in the fossil woods are indigenous.

The results listed in Table 1 also show that 4-hydroxyproline is present in wood. This amino acid has a fairly limited occurrence in nature and is found only in structural proteins such as collagen¹⁸. The cell walls of higher plants contain a hydroxyproline-rich glucoprotein, extensin¹⁹. This structural protein is probably the source of hydroxyproline and perhaps some of the other amino acids in wood. There is a gradual decrease in the hydroxyproline content of wood with age; a similar decrease is seen in fossil bones²⁰.

Enantiomeric ratios

Amino acid enantiomeric ratios for the various wood samples are shown in Table 2. In every case, the amount of racemisation increases in the order bristlecone pine < Sangoan < Acheulian. There is an unexplained discrepancy between the D/L aspartic acid ratios determined by the diastereomeric dipeptide and the gas chromatographic techniques for the Acheulian sample, but the order and the direction of increase are the same. (This is the first time we have found such a discrepancy between the two methods. This difference probably occurs because the Acheulian sample contains low quantities of the normal protein amino acids and fairly substantial amounts of unknown compounds which appear mainly in the gas chromatographic trace.) The

Table 1 Amino acid composition of modern and fossil woods ($\mu\text{mol per g dry wood}$)

	Modern cherry trunk	Modern walnut root	Bristlecone pine (TRL-63-53)	Kalambo Falls Sangoan	Kalambo Falls Acheulian
Asp	1.43	1.35	0.68	0.35	0.05
Thr	1.04	0.79	0.49	0.25	0.03
Ser	1.05	1.00	0.57	0.22	0.03
Glu	1.18	0.98	0.58	0.37	0.08
Pro	0.87	0.93	0.57	0.89	0.20
OH-Pro*	—	—	0.57	0.15	0.03
Gly	1.45	1.22	0.62	0.43	0.11
Ala	1.73	1.15	0.78	0.44	0.08
Val	1.10	0.89	0.63	0.26	0.06
Ile	0.62	0.49	0.47	0.14	0.04
Leu	1.17	0.98	0.68	0.26	0.06
Tyr	0.26	0.13	0.09		
ϕ -Ala	1.01	0.59	0.30	0.28†	0.13†
Total	12.91	10.50	6.46	3.89	0.87
Age	0	0	4,220 yr‡	> 40,000 yr§	≥ 60,000 yr

*OH-Pro values were determined by using approximate OH-Pro/Pro values from GC analysis. Values were not determined for modern woods but the amino acid analyser results indicate OH-Pro is certainly present.

†The analyser peaks for the Sangoan and Acheulian samples were integrated by machine. Since tyrosine and phenylalanine eluted very close to one another, their integrated peak areas were not differentiated. The chromatograms show that the Tyr/ ϕ -Ala ratio is the reverse of that in the younger woods. This is surprising and may be due to a larger unidentified peak eluting very close to tyrosine.

‡Sample dated by dendrochronology and radiocarbon.

§UCLA-1857, Kalambo Falls, Site A, River Face (1959) Pit 3, No. 49 (9' 9"); see ref. 15.

|| Date based on stratigraphy and ¹⁴C ages (J. D. Clark, Appendix J, ref. 15, and personal communication) Kalambo Falls, Site B (1959) Floors 6-8, No. 14.

Table 2 Amino acid enantiomeric ratios

	Asp	Thr	Ser	Glu	Pro*	OH-Pro*	Ala	Val	Ile	Leu	Phe
Bristlecone pine TRL-63-53	0.11 (0.09)†	0.016	0.022	0.065	0.049	0.031	0.044	0.020	(0.02)†	0.043	0.044
Kalambo Falls	0.13	0.016	0.026	0.13	0.28	0.057	0.10	0.052	(0.03)†	0.06	0.062
Sangoan	(0.10)†										
Kalambo Falls	0.57	(—)‡	0.078	0.48	0.60	0.101	(—)‡	0.28	(0.07)†	0.21	0.27
Acheulian	(0.21)†										

*The D and L peaks of these two amino acids were analysed by combined gas chromatography-mass spectrometry to verify the absence of interfering peaks.

†Determined on amino acid analyser at SIO. All other values were determined by gas chromatography at NASA.

‡Not possible to determine because of interfering peaks.

increasing racemisation of the amino acids with increasing age of the wood indicates that the amino acids are indeed indigenous.

Comparison of wood with bone and solution ratios

The relative racemisation rates of amino acids in wood seem to be different from those in bone and in aqueous solution². The data in Table 2 indicate that proline has the fastest racemisation rate in wood. Proline also has a fast rate in aqueous solution (J.L.B., unpublished), whereas in bone its racemisation rate is comparable to that of alanine and glutamic acid (J.L.B., K. A. Kvenvolden, and E.P., unpublished). Aspartic acid, which has the fastest racemisation rate in bone and in aqueous solution, racemises at about the same rate as, or perhaps even slower than, glutamic acid in wood. Based on the Acheulian results, isoleucine epimerisation in wood seems to be slower than the racemisation of valine and leucine. All three of these amino acids have nearly the same racemisation rates in bone and in aqueous solution. The gas chromatogram of the Acheulian sample showed numerous unidentified peaks eluting near valine, alanine, leucine and serine, and it is possible that spurious peaks caused erroneous D/L ratios for these amino acids.

Based on analyses of bones from sites in east and south Africa, racemisation in wood seems to be much slower than in bone^{21,22}, and to confirm this, high temperature kinetics experiments were carried out. Bristlecone pine samples (TRL-71-3, ~ 3,170 yr) were used in these studies, rather than modern wood. Modern woods behaved very differently during these experiments, and had high D/L aspartic acid ratios (0.16 and 0.12). Modern wood tended to stain glassware and smoke profusely during acid hydrolysis, whereas the older woods did not (probably because of the absence of sap). The sap of modern woods has been shown to contain free amino acids¹⁰, whereas free amino acids are absent in the bristlecone pine sample. The bristlecone pine behaved more like the fossil woods during these experiments. Apparently the organic substances present in the wood sap are labile and have decomposed or have diffused out of the wood after 3,000 yr.

The kinetics experiments run on bristlecone pine (TRL-71-3) showed that the epimerisation of isoleucine follows reversible first-order kinetics¹⁷. The first-order rate for conversion of isoleucine to alloisoleucine, k_{iso} , was determined at several temperatures. These results, along with the k_{iso} values in aqueous solution²³ and bone²¹, are shown in Fig. 1. The high temperature results confirm that the racemisation rates in wood are much slower than those in bone or in aqueous solution ($k_{iso, bone} \sim 10 \times k_{iso, wood}$). The Arrhenius activation energy in wood, 29.4 kcalorie mol⁻¹, is similar but slightly lower than in bone ($E_a = 33.3$ kcalorie mol⁻¹) or in aqueous solution ($E_a = 31.3$ kcalorie mol⁻¹).

Interpretation

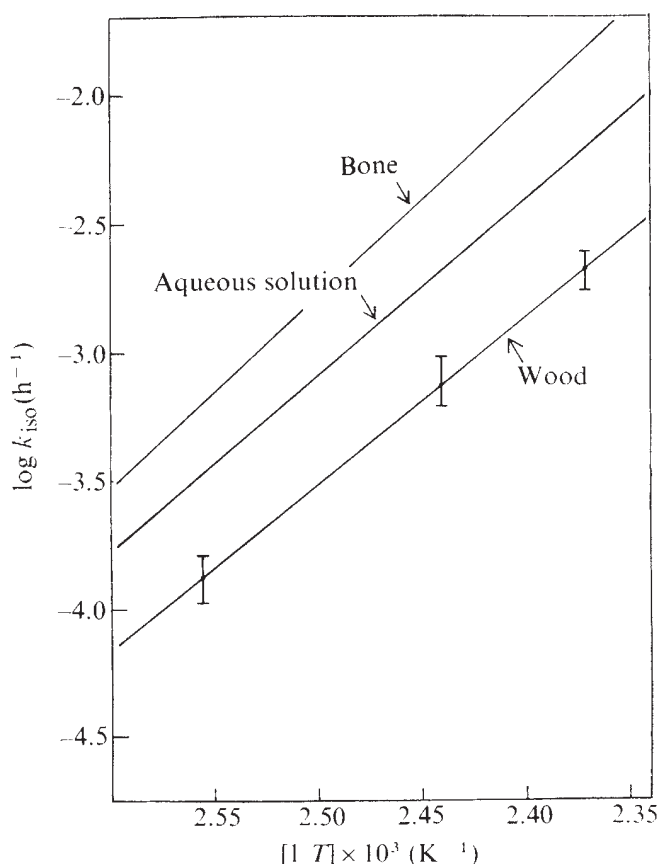
The slower racemisation rate in wood may occur because the amino acids in wood are bound in an organic matrix. Since racemisation requires the presence of water^{20,21}, a hydrophobic environment in wood could slow down the racemisation rate—

bones impregnated with tar have racemisation rates considerably slower than bones in normal geological environments¹⁶. Racemisation studies in coprolites, also an organic matrix, similarly show rates of racemisation slower than bones from the same location (J.L.B., and M. G. Petit, unpublished). One important difference between the isoleucine epimerisation kinetics in wood and those of other systems, is that the equilibrium allo/iso ratio is 0.86 in wood, compared with 1.25–1.40 for aqueous solution and bone^{20,24}. Again, this difference may arise from the organic nature of the wood.

Ages

The racemisation results we have obtained for the Kalambo Falls woods can be used to calculate an age for the upper Acheulian industry at this site. The racemisation dating technique is best applied by 'calibrating' the rate of the amino acid racemisation reaction for a particular site using the extent of racemisation in a sample which has been radiocarbon dated. After this 'calibration' has been carried out, other samples from the same site can be dated based on the extent of their

Fig. 1 Arrhenius plot of isoleucine-alloisoleucine epimerisation kinetics in wood, in aqueous solution²² and in bone²³.



racemisation. The ages of fossil bones deduced using this technique are in close agreement with radiocarbon ages and the ages deduced from other evidence^{21,22}.

Our Sangoan sample was radiocarbon dated at > 40,000 yr old. Therefore, by using the Sangoan sample to calibrate the racemisation rate in wood at Kalambo Falls, only a minimum age can be assigned to the Acheulian sample. Calculations of this minimum age can be made using the enantiomeric ratios from each of the amino acids appearing in Table 2. Certain amino acids, however, are better than others. Aspartic acid is unsuitable because of the discrepancy between the D/L ratios determined using the gas chromatographic and diastereomeric dipeptide techniques. Because of the very small extent of racemisation of valine, leucine, phenylalanine and isoleucine in the Sangoan sample, it is difficult to calculate accurate calibration rate constants. The best choices for the calibration procedure are proline and hydroxyproline. Proline is the most stable amino acid in wood, and is still present in sizeable amounts in older woods (see Table 1). Proline also has the fastest racemisation rate, according to our preliminary data. Hydroxyproline is also a good choice for use in the calibration method because it has a much different (10 times slower) rate of racemisation (epimerisation) than proline, and it is not subject to contamination problems, because of its limited occurrence in nature.

Amino acid racemisation ages and the calibration rate constants are calculated from the following equation²

$$\ln \left\{ \frac{1 + (D/L)}{1 - K'(D/L)} \right\} \Bigg|_{\text{sample}} - \ln \left\{ \frac{1 + (D/L)}{1 - K'(D/L)} \right\} \Bigg|_{t=0} = (1 + K')kt$$

where k is the first-order rate constant for interconversion of the D- and L-enantiomers of a particular amino acid and K' is the inverse of the equilibrium constant K_{eq} , which equals the D/L ratio at equilibrium². For amino acids with more than one centre of asymmetry, such as hydroxyproline, K_{eq} is slightly different from unity. Experimental determinations of K_{eq} for hydroxyproline show that the equilibrium constant in aqueous solution is 0.8 (ref. 20). Since hydroxyproline epimerisation in the fossil wood samples we have analysed, is so far from equilibrium, the value of K_{eq} makes little difference to our calculations. Using the D/L-proline and allohydroxyproline/hydroxyproline ratios determined for the Sangoan wood sample, an age of > 40,000 yr for the Sangoan, and the bristlecone pine as $t = 0$ values, the calculated racemisation rates are $k_{OH-P_{ro}} \leq 6.5 \times 10^{-7} \text{ yr}^{-1}$ and $k_{P_{ro}} \leq 6.0 \times 10^{-6} \text{ yr}^{-1}$. These values of k , combined with the D/L proline and allohydroxyproline/hydroxyproline ratios for the Acheulian wood, give ages of > 110,000 yr for hydroxyproline and > 110,000 yr for proline. The agreement of these two values is excellent,

especially considering the order-of-magnitude difference in rate constants. It should be pointed out that similar calculations using the other amino acids listed in Table 2 all give older minimum ages. The ages deduced from the other amino acids are less reliable, however, for the reasons we have discussed earlier. In any case, the combined racemisation results show that > 110,000 yr is an absolutely minimum age for the Sangoan-Acheulian transition at Kalambo Falls.

The age of the end of the Acheulian industry in Africa has sometimes been used to define the beginning of the Upper Pleistocene, a time span poorly defined by geochronometric means²⁵. (The Upper Pleistocene is more usually defined as coincident with the beginning of the Last (Eemian) Interglacial at ~ 125,000 yr (ref. 26).) Our racemisation-deduced age of > 110,000 yr at Kalambo Falls is consistent with numerous radiometric dates for older Acheulian sites in Africa²⁵, and compares well with geologically-inferred dates for the beginning of the Eemian and the end of Acheulian industry in southern Africa²⁷.

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letters to nature

UBVRI photometry of Nova Cygni 1975

MULTICOLOUR photometric observations have been made of Nova Cygni 1975. A periodic variation of ~3.2 h was detected by Tempesti¹ on 1975 September 14 when the nova was at $m_v=6$. On 1975 September 18 we made a 4-h survey in five colours, UBVRI, to check this variation using the 40-cm telescope of the Laboratorio di Astrofisica Spaziale CNR at Frascati, in the Monte Porzio Observatory. The

variation was confirmed for all colours, and other significant characteristics of the light curve have been detected.

The photoelectric photometer used, the DAPHNE system, has been described elsewhere²; its main features are: the photomultiplier used is the RCA C31034A in photon counting mode, with the GaAs photocathode cooled to -20 °C; measurements in the five bands are serial with the possibility of choice of acquisition time; and each sequence of measurements can be started with a quartz clock at a well

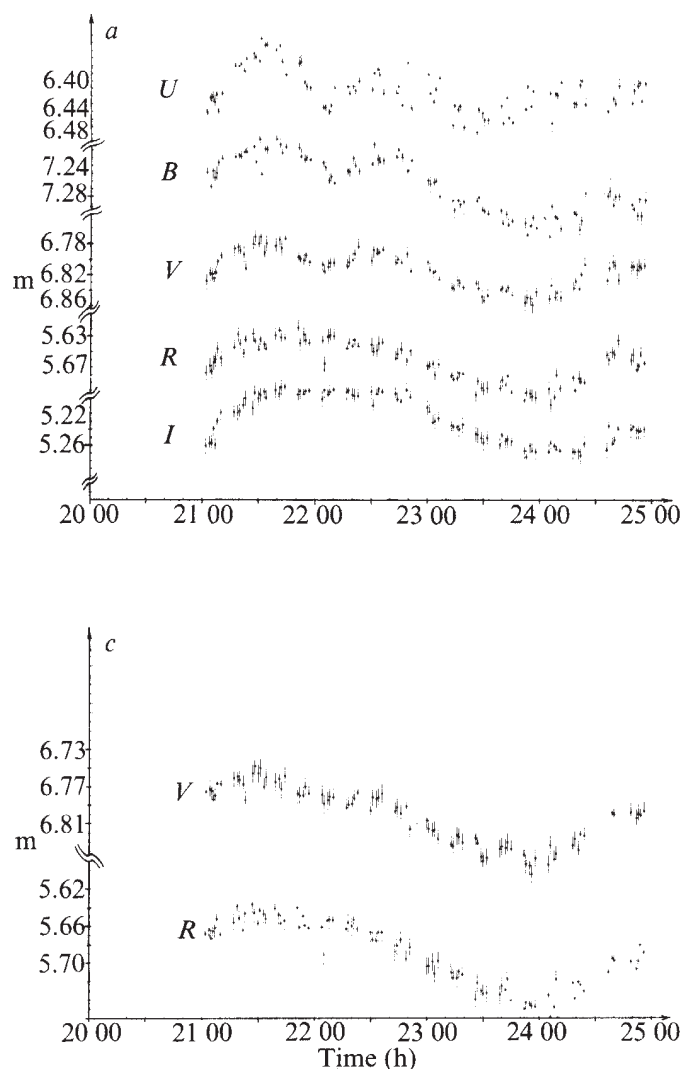
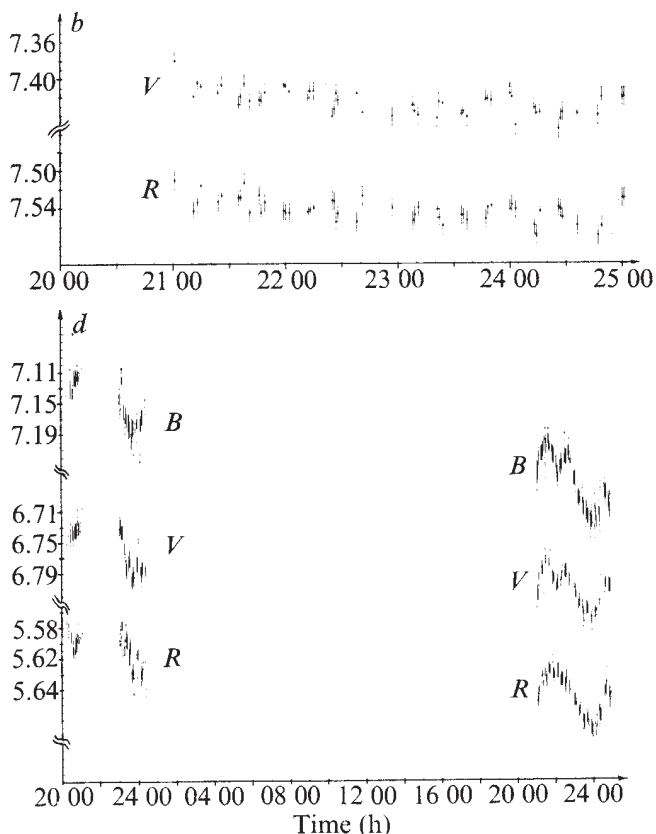


Fig. 1 *a*, Five colour, 4-h photometry of Nova Cygni 1975, on September 18, 1975. *b*, *V* and *R* photometry of the comparison star, obtained from standard stars. *c*, *V* and *R* photometry of the nova, obtained from standard stars. *d*, *B*, *V*, *R* photometry of the nova for the nights of September 17 and 18.



defined time. An electronic offset device switches the telescope from the comparison star to the nova, and vice versa; so that data from the comparison star and nova can be obtained with a minimal interval. The acquisition time was 3 s for each band, and the magnitudes in the five colours have been plotted and are shown in Fig. 1*a*.

All the light curves show a variation ~ 0.08 mag. Moreover, a new feature is present in every band: a secondary minimum appears centred at 22 h 05 min, shifted ~ 110 min from the maximum. The relative intensity of this anomalous absorption varies with colour, vanishing towards the long wavelength end.

Measurements of the comparison star, BDA473294, with respect to a set of standard stars on September 18 are shown in Fig. 1*b*. The error bar of each measurement (± 0.01 mag r.m.s.) was derived from a check on standard stars. The magnitudes in the five bands derived for the comparison star are: $U=7.49$, $B=7.48$, $V=7.43$, $R=7.54$, $I=7.69$, which have been used as a standard for computing the nova magnitudes. No periodic variation was detected, in either magnitude or colour for the comparison star. The same variation of luminosity of the nova is still obtained after applying the coefficients for the night obtained from standard star measurements (see Fig. 1*c*). This excludes any possibility of intrinsic variation in the comparison star and confirms the short time variation of the nova.

Figure 1*d* shows the results for September 17 and 18 together. The decrease of the mean intensity is evident, with a rate of almost 0.1 mag d^{-1} . Any period from 3 h 00 min to 3 h 48 min fits the two sets of measurements:

this implies either different features from night to night, or a real period $> 3 \text{ h } 48 \text{ min}$.

We are grateful to Professor P. Tempesti for prompt communication on this atypical feature of the nova.

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Coronal lines in near infrared spectrum of Nova Cygni 1975

WE have been monitoring the 2- and 3- μm spectral regions of Nova Cygni 1975 at irregular intervals. Shortly after maximum, these spectral regions were dominated by the lines of hydrogen and a few of neutral helium superposed on a continuum presumably of free-free radiation. These spectra could be quantitatively interpreted on traditional recombination theory¹, assuming a temperature of $\sim 10^4$ K. Beginning with a spectrum

obtained on September 29, 1975 a number of additional lines have appeared in the spectrum. These new lines have increased in strength so that in our most recent data they nearly dominate these spectral regions. These two phases of the spectral development of Nova Cygni 1975 are illustrated for the 2- and 3- μ m spectral regions in Figs 1 and 2.

To isolate more clearly the additional spectral features, we have subtracted the earlier data, suitably scaled, from the most recent spectra. These difference spectra, also displayed in Figs 1 and 2, demonstrate that the spectral changes result from additional lines superposed on a nearly-unaltered recombination spectrum.

The derived wavelengths and proposed identifications for these new lines are listed in Table 1. The near infrared spectrum of Nova Cyg 1975 is now dominated by forbidden lines arising from highly ionised species of Mg, Al, and Si. These lines are analogous to the coronal lines observed in the visible portion of the solar spectrum. One of them, the [Mg VIII] line at 3.03 μ m, has been previously observed in the solar corona^{2,3}. Although at first sight outlandish, these identifications appear entirely secure. None of the wavelength differences is larger than might be expected on the basis of our error in measuring the wavelength and the expected uncertainties in the predicted wave-

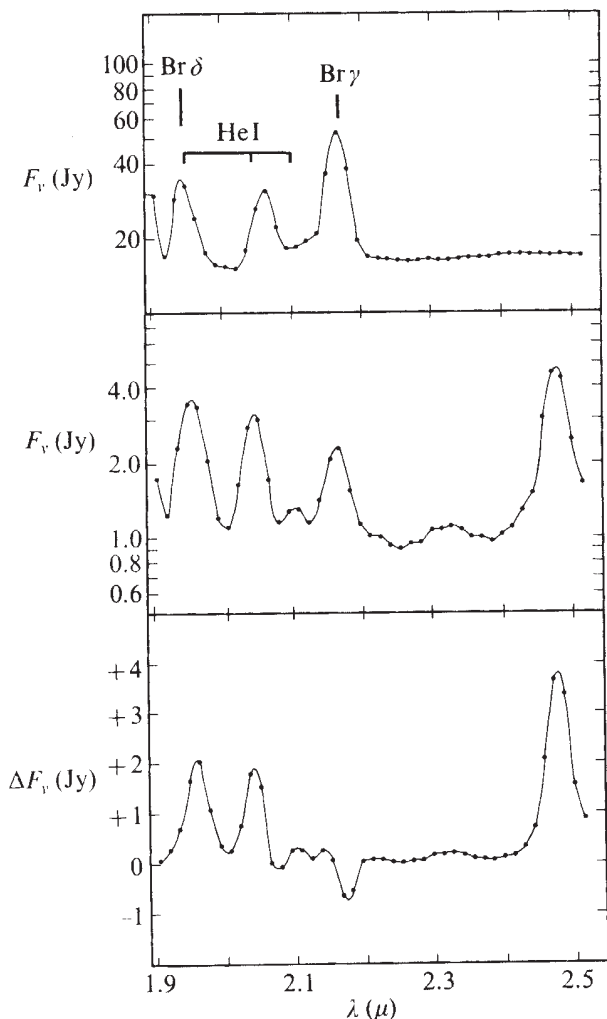


Fig. 1 The 1.9–2.5 μ m spectrum of Nova Cygni 1975 observed with an InSb detector and a circular variable interference filter having a resolution of 1.3%. The coronal lines are unresolved at this resolution. The difference spectrum in the lower panel results from subtraction of the 1975 September 12 data (top) divided by 18, from the 1975 October 27 spectrum (middle). The emission features near 2.10 and 2.14 μ m are probably artefacts of this procedure.

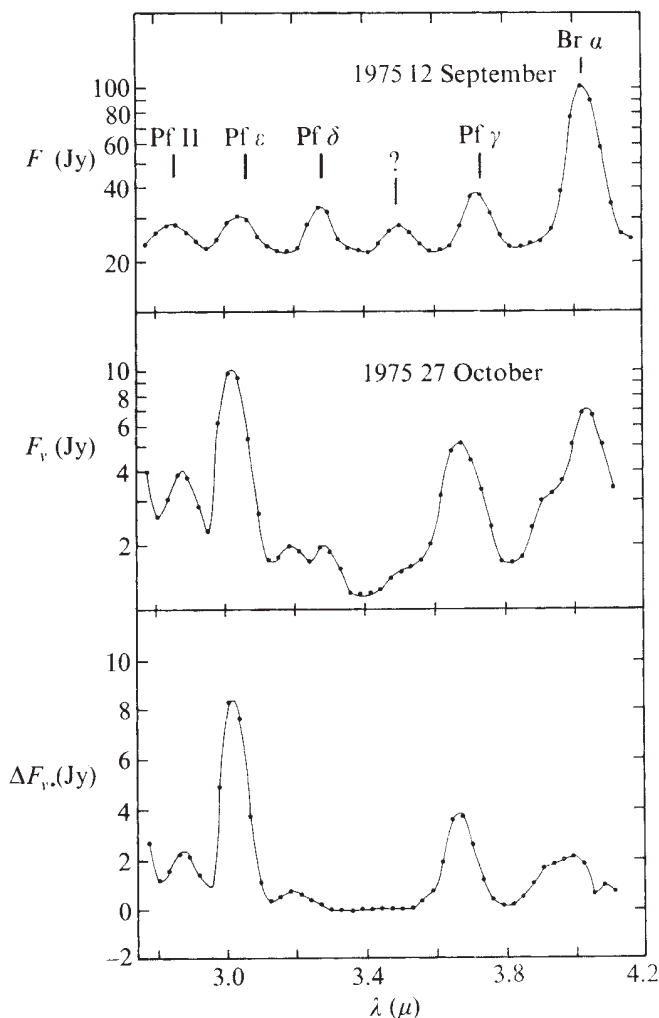


Fig. 2 The 2.8–4.1 μ m spectrum of Nova Cygni 1975 observed with an InSb detector and a circular variable interference filter having a resolution of 2.2% on the same dates as in Fig. 1. The difference spectrum was obtained in the same manner as that in Fig. 1.

lengths. Even more convincing is the fact that virtually all the coronal lines expected in these portions of the spectrum are present. Considering $\Delta J = 1$ transitions within the ground state of ions of elements whose normal abundance is $> 10^{-6}$ that of hydrogen, only two predicted lines are not definitely present. One, the line at 4.09 μ m for [Ca VII], is inextricably blended with Brackett α at 4.05 μ m. The other, at 2.29 μ m, would come from [Ca XIII] whose ionisation potential of 726 eV adequately explains its absence.

This result has a number of interesting consequences. Since these coronal lines have been increasing in absolute strength, a region with very high temperature, $\sim 10^6$ K, must be developing within the nova material. Furthermore, our data indicate that the degree of ionisation is still increasing, as the [Al IX] and [Si IX] lines have been increasing more rapidly than the other coronal lines. The visible spectrum of Nova Cyg 1975 does not yet, however, exhibit any coronal lines (J. Cohen, personal communication) although coronal lines have been observed in a number of previous novae⁴. Apparently, for reasons that are not yet clear, the near infrared provides a more sensitive indicator of the presence of a coronal zone than does the visible spectrum. This raises the possibility that the development of a very high temperature zone in novae is more common than presently deduced from visible data alone.

Table 1 Coronal line identifications in Nova Cygni 1975

$\lambda_{\text{obs}}(\mu\text{m})$	σ	$\lambda_{\text{pred}}(\mu\text{m})$	Identification		
1.959	0.007	1.963 (ref. 6)	[Si VI]	$^2\text{P}_0$	(1/2, 3/2)
2.040	0.007	2.045 (ref. 6)	[Al IX]	$^2\text{P}_0$	(3/2, 1/2)
2.32	0.02	2.323 (ref. 7)	[Ca VIII]	$^2\text{P}_0$	(3/2, 1/2)
2.474	0.007	2.481 (ref. 5)	[Si VII]	^3P	(1, 2)
2.879	0.014	2.904 (ref. 6)	[Al V]	$^2\text{P}_0$	(1/2, 3/2)
3.021	0.014	3.030 (ref. 6)	[Mg VIII]	$^2\text{P}_0$	(3/2, 1/2)
3.18	0.03	3.210 (ref. 8)	[Ca IV]	$^2\text{P}_0$	(1/2, 3/2)
3.661	0.014	3.656 (ref. 5)	[Al VI]	^3P	(1, 2)
3.72	0.02	3.700 (ref. 5)	[Al VIII]	^3P	(2, 1)
3.92	0.02	3.917 (ref. 5)	[Si IX]	^3P	(1, 0)

Finally, since a number of ionisation stages are observable for Si and Al, we may be able ultimately to deduce the physical conditions within the coronal zone. This raises the exciting prospect of being able to deduce relative elemental abundances for the high temperature component. One might expect the high temperature zone to be more intimately involved with the source of the explosion, and thus its chemical composition may contain important clues for our understanding of the nova process.

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commonly accepted value of 1.7 for the solar Fe/S ratio⁶, the only iron sulphide predicted to exist at equilibrium is stoichiometric troilite, FeS, containing <0.5 weight % Ni. It is therefore pertinent to ask why the Orgueil sulphide differs in both crystal structure and Ni content from the predictions of equilibrium condensation. Four possible reasons may be advanced.

First, the value taken for the solar Fe/S ratio may be in error. A value of <1 could result in pyrrhotite being a stable phase. Depending on the actual value of Fe/S, this pyrrhotite would either be accompanied by another sulphide (either pentlandite or pyrite) or fall compositionally within the monosulphide solid solution field. In this last case, the composition of the mineral would be exactly equal to the solar proportions of the three elements. Unless the value taken for the solar Fe/Ni ratio is also in error, in the opposite sense, such is not the case for Orgueil pyrrhotite (Fig. 1) nor does the meteorite contain any pentlandite or pyrite.

Second, stabilisation of another Fe-bearing phase could result in competition for the available Fe and consequent formation of an Fe-deficient sulphide. At around 680 K the only plausible alternative is production of fayalitic olivine from enstatite



At this temperature, however, the maximum possible FeO content of olivine in equilibrium with the nebular gas is ~5 weight %, which is inadequate to alter the course of sulphide condensation. In addition, this reaction is between two solid phases and is unlikely to compete effectively with the gas-

Formation of iron sulphide in solar nebula

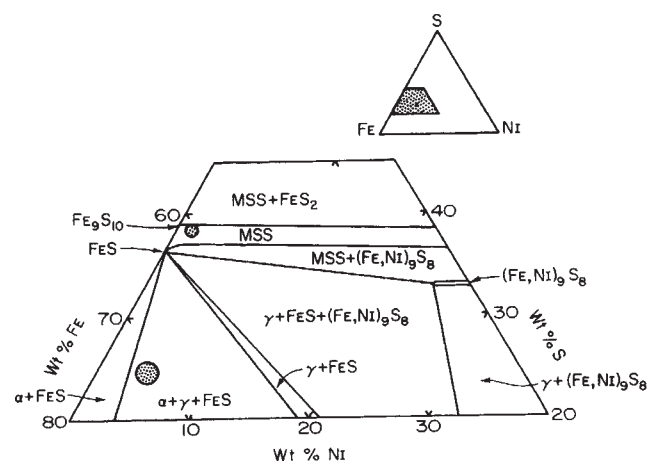
IODINE-XENON dating has established that iron sulphide in the Orgueil carbonaceous meteorite is one of the oldest known meteoritic mineral phases, and probably dates from the condensation stage of the early Solar System¹. This sulphide, although generally assumed to be troilite, FeS, is in fact the Fe-deficient monosulphide, pyrrhotite, $(\text{Fe,Ni})_9\text{S}_{10}$, containing ~1 weight % Ni (ref. 2). The purpose of this note is to suggest that such mineral chemistry is inconsistent with equilibrium condensation, and that the course of condensation may have been modified by kinetic effects.

Strictly, iron sulphide is not a condensate but a reaction product between a condensate, metallic Fe, and S in the gas phase. The reaction



goes to the right at 680 K, in a cooling nebula of solar composition³. It is necessary to consider the effect of Ni on this reaction and the course of equilibrium condensation may be determined by means of the Fe-Ni-S ternary phase diagram (Fig. 1). This shows that the solar composition lies within the kamacite-taenite-troilite stability field at 680 K. Thus, formation of troilite, which can contain no more than 0.5 weight % Ni (ref. 4), from kamacite containing 6 weight % Ni would require precipitation of taenite, with ~40 weight % Ni (ref. 4). Consequently, with the

Fig. 1 Phase relations in a portion of the Fe-Ni-S system in equilibrium with vapour at 680 K and $\ll 1$ atm pressure, based on refs 4, 5. Large circle represents solar composition⁶; small circle shows composition of Orgueil pyrrhotite². MSS is monosulphide solid solution; FeS_2 is pyrite; FeS is troilite; $(\text{Fe,Ni})_9\text{S}_8$ is pentlandite; α is kamacite, body-centred cubic Fe; γ is taenite, face-centred cubic Fe.



solid reaction. It would also result in a sulphide with a Ni/Fe ratio greater than solar, rather than less, because meteoritic olivines contain only trivial amounts of Ni.

A third possibility is that oxidation⁸ of a Ni-poor troilite condensate might have produced the observed Ni-bearing pyrrhotite, if the Ni was retained in a sulphide phase comprising a small fraction of the original S. Several reaction pathways are possible; the observed Orgueil mineralogy (pyrrhotite plus magnetite plus epsomite, MgSO₄) suggests the following representation



It is far from certain, however, that such a reaction could have occurred. If it did occur it must have been after accretion of the meteoritic parent body to retain the oxidised species of sulphur. Therefore the ¹³⁹I/¹²⁷I ratio inferred by Lewis and Anders¹ would have been characteristic of some late stage in planetary accretion rather than of the condensation stage, as they propose. Such a conclusion would impose severe constraints on models of early Solar System accretion, especially as accumulation of the other meteorite parent bodies apparently took place after the Orgueil alteration stage¹. Another argument against the oxidation hypothesis is that pyrrhotite-magnetite associations are virtually never found in Orgueil. Where alteration of pyrrhotite is found, the reaction products are sulphate, elemental sulphur and limonite (an hydrated iron oxide of variable composition).

A more reasonable possibility is that sulphide formation in the nebula was inhibited by sluggish diffusion, so that sulphur began to react with previously condensed troilite to form pyrrhotite



This reaction is also capable of explaining qualitatively the Ni content of Orgueil pyrrhotite. Initial formation of troilite would have resulted in the enrichment of Ni in the metal at the interface with the growing sulphide⁴. Conversion of this sulphide to pyrrhotite, which can accept large amounts of Ni and in which diffusion is likely to be easier than in either troilite or kamacite, would result in transfer of some Ni from metal into sulphide. The amount of such Ni would presumably have been kinetically limited.

Observations on Orgueil sulphides therefore suggest that below ~700 K the course of solar system condensation was modified by kinetic effects and that equilibrium may not have been achieved. These conclusions have implications for the Bi, Tl, In cosmothermometers which are considered to be applicable over the temperature range 420–500 K (refs 9, 10). In fact, the reactions considered here are particularly appropriate because Bi and Tl are believed to condense as alloys in metallic Fe, and In as a sulphide in troilite. If sulphur failed to reach equilibrium with Fe at 680 K, it is difficult to understand how Bi and Tl could have done so at a temperature 200 K below. Recall that, by then, all available Fe would be covered with a layer of pyrrhotite. A final detail is that the solubility data used in the thermodynamic calculations, on which the equilibrium theories are based, were for troilite rather than pyrrhotite, in which heavy trace elements are probably more soluble than in stoichiometric troilite.

Identification of Orgueil pyrrhotite as a nebular condensate is also in conflict with predictions of Blander's Constrained Equilibrium Theory¹¹, where, as a result of inhibited nucleation of metallic Fe and FeS, troilite is predicted to condense at temperatures in the range 1,050–1,450 K. At such high temperatures it is unlikely that reaction (1), once nucleated, would fail to reach equilibrium.

These conclusions apply strictly only at the formation location of the carbonaceous meteorites. In the absence of evidence to the contrary, such as, hypothetically, occurrence of primordial troilite in some other type of meteorite, they may be applied to condensation at any point in the Solar System swept by the 680 K isotherm.

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Atmospheric shock waves and condensation clouds from Ngauruhoe explosive eruptions

VISIBLE shock waves ('flashing arcs') passing through the air above a volcanic vent were first recognised by Perret¹ during explosive eruptions of Vesuvius in 1906. Although reportedly rare^{2,3} in subsequent volcanic eruptions, large visible atmospheric shock waves have accompanied explosive activity of Ngauruhoe, an andesite volcano in New Zealand, in 1954 (ref. 4), 1974 (ref. 5) and 1975.

During a series of seven highly explosive eruptions on the afternoon of February 19, 1975, observational and photographic records of eruption sequences were obtained, the most complete being of the largest explosion at 1810 NZDT which accompanied a volcanic earthquake of magnitude M_L 3.4 (J. H. Latter, personal communication). Observations were made from both Mangatepopo Track and Chateau Observatory, respectively 3.4 km and 9.2 km from, and 1,110 m and 1,170 m below, the 100-m deep funnel-shaped summit crater. Each eruption was an isolated event following a 20–60-min period during which ash emission and volcano-seismic activity declined to very low levels. Explosion initiation appeared instantaneous, heralded by a large atmospheric shock wave clearly visible as a luminous arc expanding very rapidly through thin cloud, steam and clear air to several hundred metres above the crater. Attempts to photograph the shock waves were unsuccessful. Each shock wave was closely followed by the cannon-like, high-velocity projection above the crater of a dense slug of highly compressed gas and solid ejecta. Apparently due to vent shape the eruption slug formed three expanding sub-cylindrical coalescing plumes with rounded outlines and slightly pointed tops (Fig. 1a), when 200 m above the crater rim. The first photograph of the explosion was taken at this instant, between 0.5 and 1.0 s after observation of the shock wave. Figure 1b, taken by another observer a fraction of a second after Fig. 1a, records development of a sub-spherical hollow 'condensation cloud', surrounding and partly obscuring the expanding eruption slug. The diameter of the condensation cloud at this moment was ~500 m, although it extended a greater distance above the crater than down the slopes of the volcano (by ~1.8:1). Figure 1c, taken ~1 s later by a third observer, records disappearance of the condensation cloud, and outward expansion of the eruption slug to envelop the summit, while large blocks (up to 20 m across) were projected through it. Eight-millimetre cine film (18 frames s⁻¹) taken of the explosion between photographs 1b and c, records the base of the condensation cloud 170 m above the crater (first frame), with its top extending into atmospheric cloud 580 m above the crater. The base of the condensation cloud travelled upwards at about 190 m s⁻¹ to reach 220 m above the crater

in the sixth frame of the film, before disappearing entirely in the seventh frame, exposed ~ 0.4 s after the first.

The audible shock wave reached the Mangatepopo observation point as a very sharp and intensely loud 'crack' arriving 10.6 s after observation of the visible shock wave (G. T. Hancox, personal communication). This is equal within limits of error to the theoretical sound travel time of 10.58 s (assuming a sound velocity of 338 m s^{-1} in still air at 10°C).

The atmospheric shock waves from each explosion were recorded as air-ground coupled waves at the Chateau seismograph 8.6 km from the crater. Air shock waves were also recorded on a nearby barograph. Each explosive pulse (up to

Fig. 1 Sequential photographs of the 1810 NZDT eruption, February 19, 1975, taken by three observers reacting to the appearance of the preceding shock wave. *a*, Eruption slug projected above crater; *b*, expanding eruption slug surrounded by 'condensation cloud'; *c*, disappearance of condensation cloud. Photographs from Mangatepopo Track by G. T. Hancox, D. M. McVicker, and R. Foley.



2.5×10^2 Pa above ambient in the 1810 eruption) was accompanied by a lesser fall (to 1.5×10^2 Pa below ambient) in the following rarefaction phase. The explosions were heard more than 80 km from the volcano.

Short lived condensation clouds similar to those which formed at Ngauruhoe accompany nuclear explosions, an example being particularly clearly illustrated in photographs of the 1946 Bikini underwater explosion^{6,7}. Formation of the cloud is due to rapid condensation of atmospheric water vapour in the adiabatically cooled rarefaction phase which is developed behind the positive overpressure phase of the shock wave. Subsequent re-evaporation and disappearance of the cloud occurs as atmospheric pressure returns to ambient (and temperature to slightly above ambient) behind the rarefaction phase⁶. The development of such condensation clouds does not appear to have been previously recorded in volcanic explosions. They were not recognised by scientific observers during the Ngauruhoe eruptions, and their existence was only realised after study of the photographs.

The shape of the 1810 NZDT condensation cloud (Fig. 1*b*) indicates that the initial near-planar shock wave within the vent had diffracted around the crater rim to produce a subspherical blast wave. The departure from sphericity of the cloud also suggests that if the shock moving down the cone had slowed to sonic speed (as seems likely from crater geometry and the measured sound travel time) the shock velocity directly above the crater was at least 1.8 times greater at Mach number $M = 1.8$, or $\sim 600 \text{ m s}^{-1}$.

An estimation of the initial velocity of the 1810 NZDT ejecta slug is hindered by lack of accurate timing of the first photographs, the limits of 0.5–1.0 s after explosion initiation indicating an average ejecta slug velocity ranging between 300 and 600 m s^{-1} over the first 300 m. Several methods of determining initial ballistic ejecta velocities from maximum range within the atmosphere have been developed, most recently by Wilson⁸ who allowed for an increased drag coefficient (C_d) at high Mach numbers. During the Ngauruhoe eruptions, a 0.8 m diameter lava block of density 2.5 g cm^{-3} was thrown 2.8 km, landing 980 m below the vent. Assuming a horizontal range of 2.5 km, a minimum initial velocity approaching 400 m s^{-1} is interpolated from Wilson's tables. That this is of the correct order may be indicated by the relationship from one-dimensional shock tube theory⁹

$$U = \frac{u_p}{2-2\mu^2} + \left(c^2 + \frac{1}{4} \left(\frac{u_p}{1-\mu^2} \right)^2 \right)^{1/2}$$

where U = shock velocity, u_p = piston velocity, c is velocity of sound, and $\mu^2 = 1/6$ for air. A minimum initial Ngauruhoe shock velocity of 600 m s^{-1} (as suggested by condensation cloud shape) would be associated with a piston (that is, ejecta slug) minimum velocity of about 340 m s^{-1} . Similar velocities ($> 330 \text{ m s}^{-1}$) have been observed for venting gases and ejecta in the Sedan underground thermonuclear explosion¹⁰, and considerably higher velocities ($600\text{--}660 \text{ m s}^{-1}$) have been calculated for ejecta from explosive eruptions of Arenal, a volcano in Costa Rica^{8,11}.

For cannon-like volcanic explosions, McBirney¹² has derived the equation $P = 0.0125v^2$, where P is the explosion pressure (atm) and v is ejecta velocity (m s^{-1}). Assuming an initial ejecta velocity of 400 m s^{-1} , a minimum explosion pressure of 2×10^8 Pa is calculated for Ngauruhoe. McBirney also deduces that such high pressures cannot be generated by the exsolution of volatiles from magmas of normal water content, but, instead, are caused by the rapid heating of confined meteoric water to near magmatic temperatures. This model it appears can be applied to Ngauruhoe, where a lava column rising within the volcano may rapidly heat pockets of meteoric water confined beneath the solid lava of an earlier formed plug⁵. High pressures develop until the mechanical

strength of the confining rocks is exceeded, and catastrophic failure occurs. Intense steam explosions result, with mechanism perhaps similar to that envisaged by Bennet¹³. At Ngauruhoe, the almost instantaneous release of pressures apparently $> 2 \times 10^8$ Pa, at supersonic velocities, is clearly adequate to generate large visible shock fronts above the volcano.

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Temperature limits on the early Archaean ocean from oxygen isotope variations in the Isua supracrustal sequence, West Greenland

FROM a reconnaissance survey of the oxygen isotope composition of a sequence of metamorphosed sedimentary rock units at Isua, West Greenland, it is possible to set wide temperature limits of ~ 0 – 146°C on the early Archaean ocean.

The study area, part of the Greenland Archaean terrain, is 150 km north-east of Godthaab. The Isua supracrustal sequence, which consists of conglomerate, quartzite, acid metavolcanics, garnet-staurolite schist, banded iron formation, and laminated chert and carbonate interlayered with mafic flows or sills, may have been deposited in shallow water¹. This sequence is enclosed by gneisses (Fig. 1) that range in composition from granodiorite to granite, and both the supracrustal sequence and gneissic terrain have been metamorphosed to amphibolite facies. A Pb–Pb age for the supracrustal sequence of $3,760 \pm 70$ Myr has been determined by Moorbath *et al.*² from whole rock and from mineral separates of the banded iron-formation while an age of $3,700 \pm 100$ Myr has been reported³ for the gneissic terrain.

Oxygen isotope analyses were performed on laminated and massive cherts and on quartz separates from banded iron formations. Oxygen was liberated from 10–20-mg samples by bromine pentafluoride and converted to CO_2 for mass spectrometric analysis⁴. Analyses were performed on a 15-cm, 60° sector mass spectrometer with a double inlet and a double collector.

The $\delta^{18}\text{O}_{\text{SMOW}}$ values⁵ for samples from three areas at Isua are presented in Table 1. Area one is the main iron formation occurrence at Isua^{2,6}. δ -values of laminated and massive chert units range from $+16.1$ – $+20.3\%$, the latter being the largest δ -value reported for a precipitated Archaean siliceous sediment. The massive chert units in the area of the main iron-formation occurrence at Isua seem to have undergone limited rehomogenisation; therefore, the ratios may closely approximate the initial ratio of these units. Quartz separates from the banded iron formations in Area One range from $+12.6$ – $+15.9\%$, showing a probable re-equilibration of the quartz with magnetite during metamorphism. In comparison, as a result of meta-

morphic effects, cherts and quartz separates from Areas Two and Three are depleted in ^{18}O as much as 10.3% compared to the maximum value found in sample 4-27/8-74 of Area One.

To interpret the data in Table 1, we have made four assumptions concerning the early Archaean ocean. First, the hydrosphere and atmosphere derived from volcanic emissions during the initial degassing of the earth as suggested by Fanale⁷. The water outgassed during this process should have a maximum δ -value of approximately $+6\%$, as a result of high temperature equilibrium with igneous material. Second, oceanic water was cycled through parts of the oceanic crust allowing for isotopic exchange reactions with igneous material at high temperatures. Third, the δ -values and environmental conditions of the early Archaean ocean are reflected in the δ -value of the precipitated sediment, and are preserved in the rock units having the highest reported $\delta^{18}\text{O}$ values. Finally, the mass of the Archaean ocean and the surface area of the earth, including the hydrosphere, were comparable to present day values.

Although the Isua cherts could have been precipitated under a number of different sets of conditions and still produce the same δ -values, some realistic constraints may be placed on the palaeotemperature of the early Archaean ocean. A minimum temperature of approximately 0°C can be established for the Archaean ocean. The approximate lower limit is 0°C , since water must remain in a liquid state for precipitation of sediments. Different maximum temperatures can be achieved by assuming different initial δ -values for the ocean. An upper temperature limit may be determined from the chert–water fractionation equation⁸

$$\Delta_{\text{cw}} = \frac{3.09 \times 10^6}{T^2} - 3.29$$

In the following, two different initial oceanic ratios are considered.

First, the Archaean ocean is assumed to have a δ -value of

Table 1 Oxygen isotope composition of chert and quartz from banded iron formations in the Isua Supracrustal belt

Sample	$\delta^{18}\text{O}_{\text{SMOW}}$	Remarks
Area One: main iron formation and associated cherts at Isua		
1-27/8-74	$+19.6 \pm 0.01$ (2)	Laminated metamorphosed chert
2-27/8-74	$+16.1 \pm 0.08$ (3)	Laminated metamorphosed chert
3-27/8-74	$+17.7 \pm 0.04$ (2)	Metamorphosed chert
4-27/8-74	$+20.3 \pm 0.13$ (2)	Metamorphosed chert
4.1-27/8-74	$+20.2 \pm 0.11$ (2)	Metamorphosed chert
4.5-27/8-74	$+19.8 \pm 0.09$ (2)	Metamorphosed chert
5-27/8-74	$+19.1 \pm 0.03$ (2)	Metamorphosed chert
7-27/8-74	$+12.6 \pm 0.02$ (2)	Quartz from banded iron formation
8-27/8-74	$+13.1$ (1)	Quartz from banded iron formation
9-27/8-74	$+15.9 \pm 0.04$ (2)	Quartz from banded iron formation
EP-11-74	$+19.1 \pm 0.03$ (2)	Metamorphosed chert
Area Two: peninsula in Lake Imarsuaq at southern tip of south-eastern limb		
EP-17-74	$+13.8 \pm 0.04$ (2)	Quartz from banded iron formation
EP-19-74	$+13.9$ (1)	Quartz from banded iron formation
EP-21-74	$+13.8 \pm 0.02$ (2)	Metamorphosed chert
Area Three: chert sequence from the north-western limb		
EP-34-74-2	$+13.5 \pm 0.15$ (3)	Metamorphosed chert
EP-34-74-3	$+13.2 \pm 0.04$ (2)	Laminated metamorphosed chert
EP-34-74-4	$+13.5 \pm 0.02$ (2)	Metamorphosed chert
EP-34-74-5	$+13.6 \pm 0.05$ (2)	Metamorphosed chert
EP-34-74-6	$+12.8 \pm 0.15$ (3)	Metamorphosed chert
EP-34-74-7	$+10.0 \pm 0.15$ (3)	Metamorphosed chert
EP-34-74-8	$+11.7 \pm 0.09$ (3)	Metamorphosed chert
EP-34-74-11	$+12.0 \pm 0.10$ (2)	Metamorphosed chert

The numbers in parentheses are the number of determinations per sample.

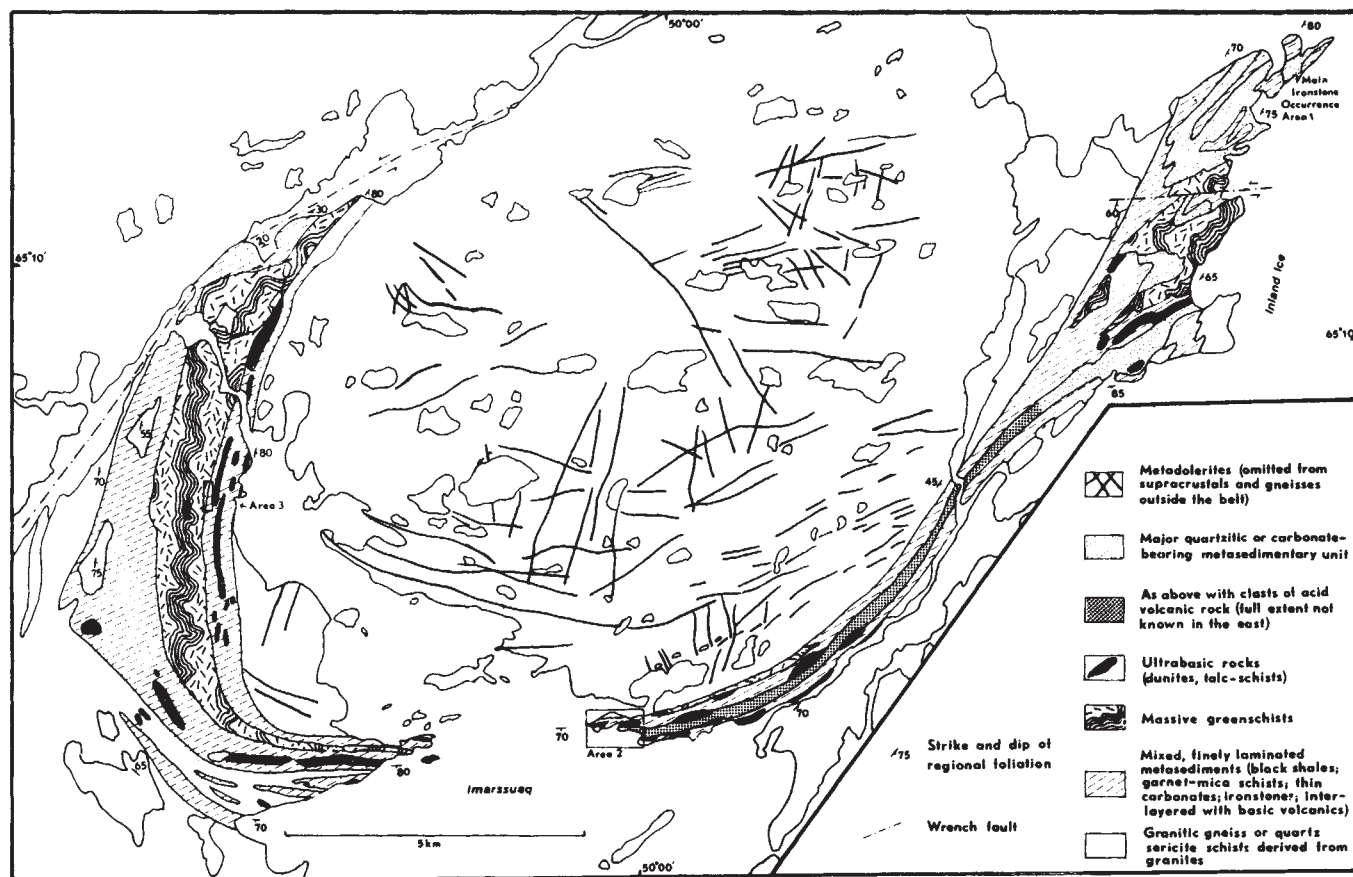


Fig. 1 Generalised geological map of the Isua supracrustal belt, West Greenland. Modified after Bridgwater and Fyfe⁶.

+6‰, the maximum for volcanic water in equilibrium with basalt at high temperatures. Isotopic fractionation between the Archaean ocean and the Isua chert with the maximum $\delta^{18}\text{O}$ value (+20.3‰) is 14.3‰. This value is a minimum fractionation, and therefore, the corresponding temperature of 146 °C from the fractionation equation is an absolute maximum for the Archaean ocean. At this temperature, the partial pressure of H_2O in the atmosphere would be 4.65 atm; and 1.62% of the water in the present ocean would be required to produce this pressure.

In the second case, the δ -value of the ocean is assumed to be 0‰, a value which has remained invariant with time. In these conditions, the ocean temperature would be ~ 89 °C and the partial pressure of water in the atmosphere equivalent to about 0.25% of the mass of the present ocean. Neither case 1 nor case 2 places any unreasonable mass deficiencies on the early Archaean ocean.

Another way (case 3) of achieving the observed δ -values is for the Archaean ocean to have had the same temperature as now but with a δ -value of -14‰. This assumes that the oceanic

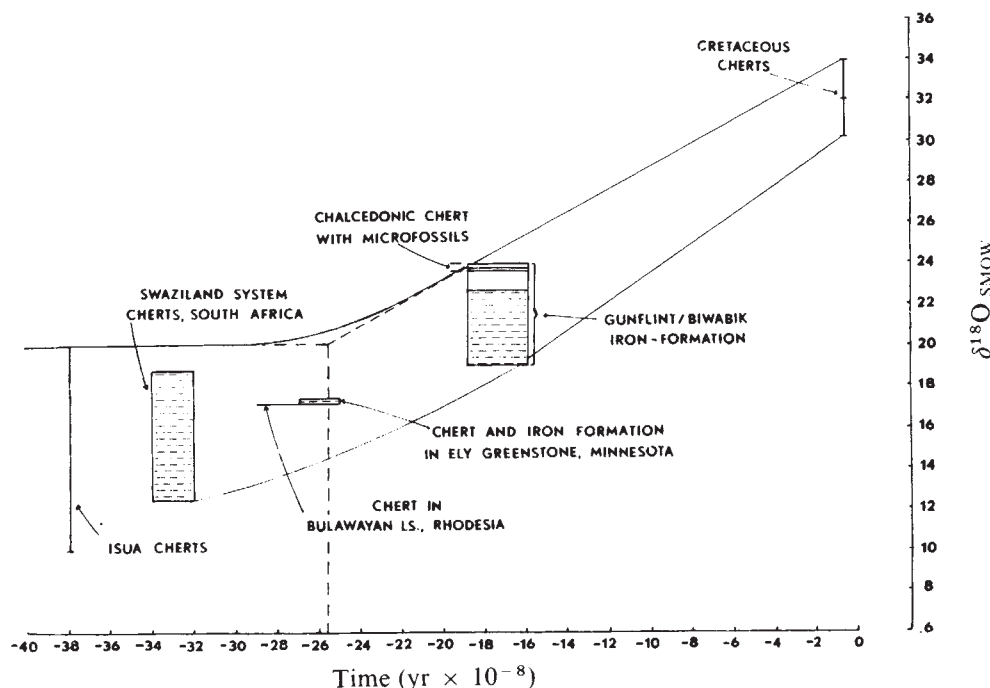


Fig. 2 $\delta^{18}\text{O}$ variations in chert-bearing units as a function of age. Cretaceous cherts, Degens and Epstein (1962); Precambrian units except Isua cherts, ref. 9; Isua cherts, this study.

isotopic composition through time has paralleled the maximum values of the chert data of Perry and Tan⁹, but has been displaced by -34% .

Irrespective of the initial ocean δ -value, processes affecting the oceanic system have occurred, allowing the establishment of the present day value of 0% . If the early ocean had a δ -value of $+6\%$ as assumed in case 1, the most important mechanism lowering the oceanic δ -value would be large scale, low temperature sedimentary processes. Low temperature sedimentation would allow for preferential fractionation of ^{18}O to the sediment, which in turn would deplete the oceans. In a hot or warm Archaean ocean (case 1 and 2) the importance of sedimentary processes in controlling $\delta^{18}\text{O}$ of the ocean would be enhanced relative to other processes. To maintain the ocean at 0% since the early Archaean as in case 2, long term buffering of the oceans by basic rocks would be necessary¹⁰. The only process that would account for a rise in the δ -value of the ocean for -14 to 0% would be the active interaction of ocean water and igneous rock reservoirs at high temperatures through some type of cycling process¹¹.

Figure 2 is a plot of age against $\delta^{18}\text{O}$ of the Isua cherts and younger metamorphosed chert units showing an apparent decrease in ^{18}O with increasing age to approximately 2,600 Myr. There is a constancy in the apparent maximum δ -value of the Archaean chert bearing units. The change in slope at $\sim 2,600$ Myr may reflect a variation in oceanic temperature coupled with changes in the mechanism which controlled the $\delta^{18}\text{O}$. These changes may have arisen from variations in the interactions between the oceans and the upper mantle. Evidence has been presented by Veizer¹² from ^{87}Sr data implying a decrease in the amount of water cycled through crustal igneous reservoirs and/or increased sedimentation. The time at which the change of slope occurs is sensitive to small variations in the maximum value of $\delta^{18}\text{O}$. A further study of the Isua area is under way to confirm the range of δ -values of the Isua cherts thus allowing for further and more thorough interpretation.

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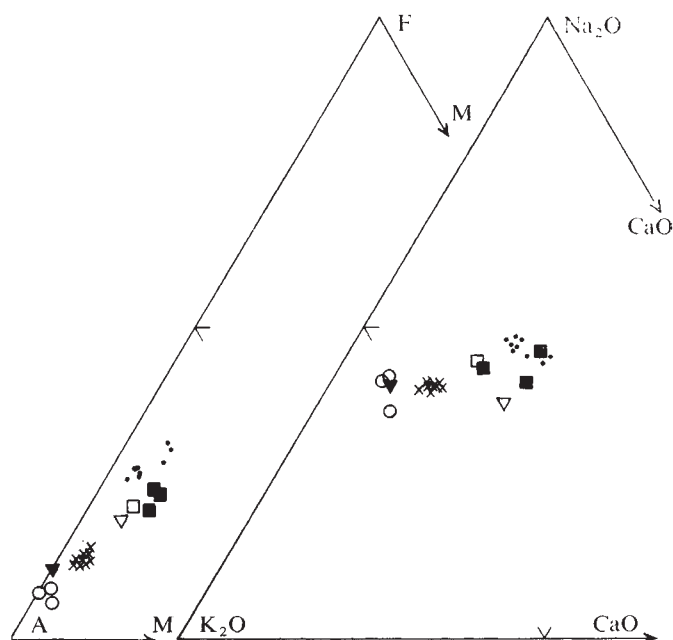


Fig. 1 Plots of chemical analyses of Quaternary pumice from various volcanoes in the Aegean. A, $\text{Na}_2\text{O} + \text{K}_2\text{O}$; F, total iron as FeO ; M, MgO . •, 1470 bc. Santorini; □, pumice 'G', Nisyros; ■, pumice 'A', Nisyros; ▽, pumice 'C', Yali; ▼, pumice 'D', Yali; ×, plateau pumice, Kos, Kalymnos, Pserimos and Telos; ○, Kefalos pumice, Kos.

The pumice on Melos occurs in a large ring in the south of the island. The ring, which is built up of repeated units of pyroclastic surge deposits, shows only slight erosion and therefore probably dates from the late Quaternary.

On Santorini, seven layers of pumice occur in the caldera walls at Kap Alai¹. The eruptions which produced the two largest deposits, consisting of air-fall pumice and ashes, and pyroclastic flows, have been dated at 18000 bc and 1470 bc². The older pumice deposit is locally >50 m thick. Isopach maps³ of the products of the younger eruption suggest that the $0.01 T_{\text{max}}$ isopach, that is the 5.5 cm isopach, must cover several thousand km^2 , and the eruption was thus of plinian type⁴. The isopachs are extended along a dispersal axis south-east of Santorini, and ash from this eruption has been identified from deep-sea cores from this area⁵.

In the Dodecanese, the products of eight pumice eruptions from three island volcanoes can be distinguished. Several pyroclastic deposits occur on Kos, and the least altered of these, here called the Kefalos deposit, occurs around the rhyolite dome of Mt Xení. The deposit consists of repeated thin pyroclastic surge and non-welded ignimbrite flow units and covers $>9 \text{ km}^2$. It is overlain by the products of a much larger eruption, including pyroclastic surge deposits, ignimbrites, and associated mud-flows. This plateau deposit covers the central and western parts of Kos and is also found on the islands of Kalymnos, Pserimos and Telos, respectively 18 km north, 8 km north, and 50 km south of Kos⁶. On these islands the plateau deposit is confined to valleys and bays facing Kos. The products of this eruption must cover $>2,500 \text{ km}^2$.

On Yali, 2 km south of Kos, there are two pumice fall deposits. The older one, 'D', is >180 m thick, built up of reversely-graded units dipping steeply north-east, and is possibly the remnant of a pumice ring. This is covered by a second fall deposit, 'C', which is 3 m thick. Between these two, an horizon of marine shells occurs at 170 m above sea level, showing that considerable vertical movements have taken place.

Multiple sources of pumice in the Aegean

QUATERNARY pumice has been produced in at least ten volcanic eruptions in the southern Aegean, from volcanoes on the islands of Melos, Santorini, and various of the Dodecanese. This multiplicity of sources has not been generally recognised before, but it should be taken into account in submarine core sampling.

Table 1 Percentage weights of phenocryst minerals in the Aegean pumices and refractive indices (r.i.) of glasses

	Phenocrysts (weight %)	Quartz (weight %)	Feldspar (weight %)	r.i.	Biotite (weight %)	Magnetite (weight %)	O'px. (weight %)	C'px. (weight %)	Refractive index of glass
Santorini (1470 BC)	10		82	1.553			16	2	1.509
Nisyros (pumice 'G')	10		80	1.553		2	17	1	1.492
Nisyros (pumice 'A')	24		83	1.548		3	13	1	1.497
Yali (pumice 'C')	2		✓	1.546					1.492
Yali (pumice 'D')	1	✓					✓		1.497
Plateau pumice, Kos	26	71	10	1.540	18	1			1.492
Kefalos pumice, Kos	8	98	2	1.540					1.501
Melos	4	92	5	1.548	2	1			1.492

✓, Present in small quantities; not measured.

Pumice from four eruptions has been preserved on Nisyros island, ~2 km south of Yali. The oldest is a pumice fall deposit exposed near the base of a cliff section within the caldera. The second fall deposit, 'G', which is rich in lithic fragments including metamorphosed limestones and plutonic rocks, is preserved mainly in the valleys on the south-west side of the island. The youngest deposit, 'A', is a pumice fall more than 20 m thick on the north-east side of the island. Pyroclastic flows which are exposed above 'G' along the north-east coastal section, seem to be the products of a fourth eruption. Fall deposits 'A' and 'G' are of plinian type.

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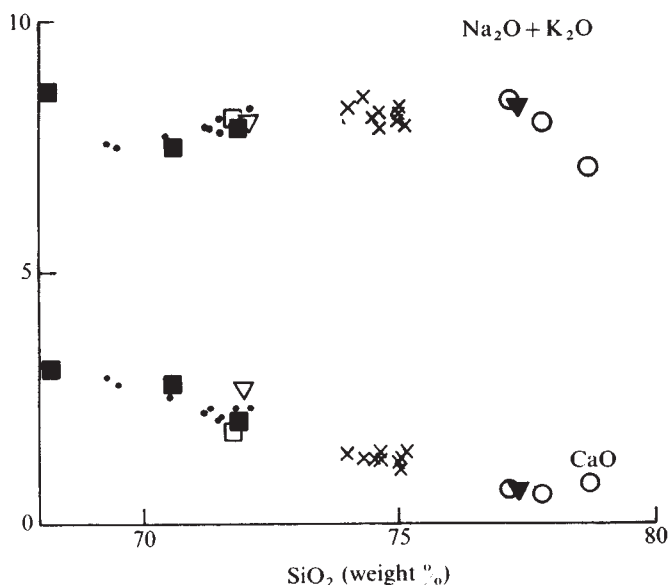


Fig. 2 Plots of CaO and Na₂O+K₂O against weight % SiO₂ for the same analysed samples as in Fig. 1; analyses recalculated to 100% water-free. The analyses cover the range dacite-rhyodacite-rhyolite-leucorhyolite.

Ocean rise-like basalts within the Galapagos Archipelago

THE Galapagos Archipelago, Iceland, and other oceanic islands, have been interpreted as surface manifestations of mantle plumes¹⁻³. A key element of this model of island development centres on the petrological and geochemical contrasts between suites of lavas erupted from the proposed mantle plumes and at oceanic rises¹⁻³. But the recognition of basalts of ocean rise composition on Iceland has prompted modification of models of petrological processes in mantle plumes^{4,5}. We know of only one other reported occurrence of ocean rise-like basalts erupted on islands of proposed plume origin: flows in the Pololu Series on the Island of Hawaii⁶.

Here we report a newly discovered occurrence of ocean rise-type basalts erupted on Santiago Island, Galapagos. The presence of these distinctive rocks is an important factor in interpreting the possible roles of mantle plume activity or other tectonic forces in the development of the Galapagos Archipelago.

Ocean rise basalts are typified by consistently low abundances of incompatible elements (such as, K₂O, TiO₂, and P₂O₅), (refs 3, 7 and 8). Most ocean rise basalts are devoid of clinopyroxene phenocrysts, but commonly contain olivine and/or plagioclase phenocrysts^{3,8}. Some pyroxene phenocrysts have, however, been observed in samples dredged from the rift valley of the Galapagos Rise^{2,9}. The compositional variation among ocean rise basalts, though quite small, has been attributed to fractional crystallisation^{7,9}, to varying degrees and depths of partial melting⁸, and to differences in the compositions of parental material¹⁰.

The same mechanisms have also been proposed as the source of more pronounced differences between ocean rise basalts and plume-derived lavas^{3,11,12}. The consistently low abundances of incompatible elements in ocean rise basalts contrast with the much greater compositional and petrographic variation and generally higher abundances of these elements in lavas erupted on oceanic islands of proposed plume origin.

The steep slopes and small size of Yali and Nisyros make it difficult to construct isopach maps of the pumice deposits. From the considerable thickness of deposits 'D' and 'A' it seems probable, however, that ashes from these islands, as well as from Santorini and Kos, should be widely found in cores from the south-east Aegean.

Major element X-ray fluorescence analyses have been made on pumice from the main eruptions to characterise these deposits. The plots of these analyses (Figs 1 and 2) illustrate the principle chemical differences. The refractive index of the glass, and the percentage and type of phenocryst minerals within the pumices have also been determined (Table 1) as an aid to the identification of the pumice from the various eruptions.

Table 1 Major element analyses of selected basalts from the Galapagos Islands and the Galapagos Rise

	1*	2	3	4	5	6	7	8
	JL-74	JL-122	JH-86	JH-115	Ave-K	Ave-A	Ave-T	Ave-D7
SiO ₂	46.80	47.06	46.46	46.87	46.75	46.81	48.36	49.60
TiO ₂	1.84	1.37	1.26	1.43	1.43	2.49	2.65	1.02
Al ₂ O ₃	16.22	16.71	16.00	16.42	16.46	15.77	14.53	15.97
Fe ₂ O ₃	1.84	2.65	2.95	3.81	2.58	3.62	3.32	
FeO	8.77	7.62	7.37	7.01	7.82	8.58	8.79	8.76
MnO	0.19	0.18	0.18	0.18	0.18	0.20	0.18	0.15
MgO	8.18	9.50	10.79	9.52	9.58	7.74	6.63	7.83
CaO	12.47	12.52	11.97	11.74	12.19	10.28	11.45	12.94
Na ₂ O	2.36	2.45	2.28	2.38	2.42	3.12	2.69	2.43
K ₂ O	0.07	0.03	0.06	0.15	0.07	0.44	0.36	0.08
P ₂ O ₅	0.11	0.07	0.10	0.12	0.10	0.32	0.25	0.07
H ₂ O ⁻							0.17	
H ₂ O ⁺	0.35†		0.27†	0.06†	0.17†	0.22†	0.61	
Total	99.20	100.16	99.71	99.68	99.75	99.59	99.99	98.95
FeO*/MgO	1.27	1.05	0.93	1.10	1.06	1.53	1.78	1.12

*1-4, Representative low-K₂O lavas from Santiago Island; 5, average analysis of 8 low-K₂O lavas from Santiago Island; 6, average analysis of 17 alkalic lavas from Santiago Island; 7, average Galapagos tholeiite, from ref. 13; 8, average analysis of 4 basalts dredged from the Costa Rica Rift Zone, Galapagos Rise, based on data from ref. 9.

†Total H₂O loss on ignition at 500 °C.

Petrographically and compositionally, the low-K₂O lavas erupted on Santiago are essentially identical to olivine tholeiites sampled from the East Pacific Rise, the Galapagos Rift and other active spreading centres (Table 1 and Fig. 1). The Santiago lavas contain up to 10% phenocrysts of olivine and plagioclase in a matrix of plagioclase laths, clinopyroxene, olivine and opaques. No pyroxene phenocrysts were observed in the eight samples we examined.

The ocean rise-type lavas from Santiago are characterised by much lower abundances of the incompatible elements and higher MgO, CaO and Al₂O₃ than either average Galapagos Island tholeiite¹³ or the more alkalic lavas also found on Santiago (Table 1). Variation diagrams of selected depleted elements against the ratio FeO*/MgO (ref. 14) show that the low-K₂O lavas are clearly disjunct from suites of other Galapagos Island rocks (Fig. 1).

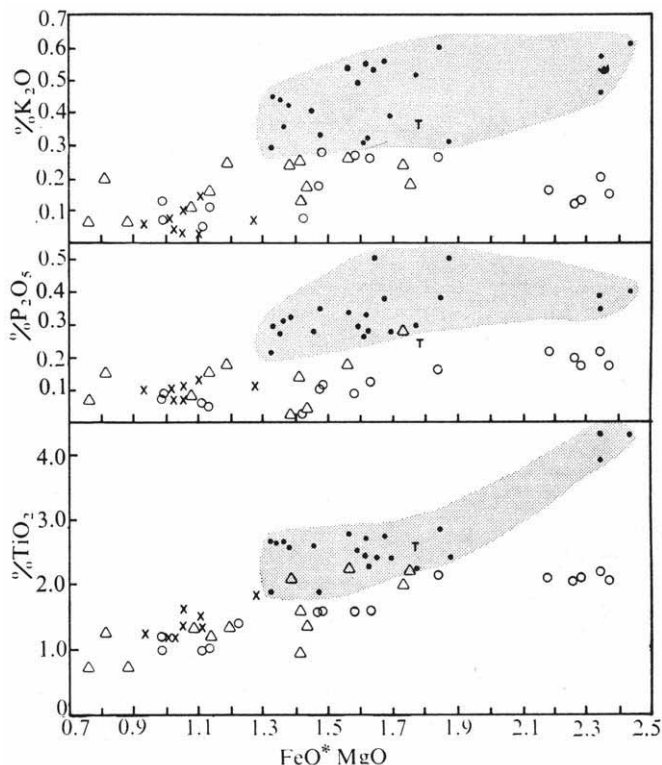
Several petrological features of the ocean rise-type basalts on Santiago indicate that they are the product of a distinct process taking place beneath the central area of the Galapagos platform. The low-K₂O lavas are younger than or coeval with the alkalic rocks which form the bulk of the exposed portion of Santiago. This is the reverse of the typical tholeiitic-alkalic lava sequential history of Hawaiian volcanoes¹⁵. Furthermore, it is unlikely that the ocean rise-type basalts are genetically related to the older or contemporaneously erupted alkalic lavas by any sort of crystal fractionation process^{3,10,16}.

Structural features associated with the ocean rise-type lavas suggest that these petrological features are related to a relatively young phase of tectonic activity in the central and eastern portion of the Galapagos Archipelago. The low-K₂O lavas on Santiago were erupted from fissures which are part of an easterly trending structural pattern of aligned vents, normal faults and fissures, and uplifted submarine lavas observed on seven islands in the central and eastern portion of the archipelago (Fig. 2)¹⁷. This structural pattern postdates the construction of the principal volcanoes forming Santiago, Santa Cruz and San Cristobal. On islands where the structural features can be dated, their ages are less than about 0.5 Myr. The ocean rise-type lavas on Santiago were erupted over the past several hundred thousand years and as recently as 1906.

The fault patterns and uplifted lavas record uplift and north-south distention in a zone extending eastward from the centre of the archipelago¹⁷. This zone straddles an east-west trending negative gravity anomaly¹⁸. Although scanty data preclude an unambiguous explanation of the

gravity low, it has been interpreted as the result of an elongate east-west curtain of mantle upwelling beneath the archipelago^{18,19}. Such a zone of upwelling would parallel the zone of active seafloor spreading at the Galapagos Rift 150 km to the north (Fig. 2)²⁰. The close spatial and temporal relationship among the subaerially erupted, ocean rise-type basalts on Santiago, the zone of uplift and north-south distention, and the negative gravity anomaly, suggest there has been a relatively recent (0.5 Myr BP to present) period of mantle upwelling beneath the central and eastern portion of the Galapagos Archipelago. Perhaps these features signal incipient rifting of the Galapagos Platform

Fig. 1 Abundances of K₂O, P₂O₅ and TiO₂ plotted against the ratio FeO*/MgO for basalts from the East Pacific Rise^{2,8}. Δ , Galapagos Ridge rift valley⁸; $\circ$, Galapagos Island average tholeiite¹³ (T), Santiago Island low-K₂O lavas ($\times$), and alkalic lavas ($\bullet$ and shaded area), (H.W.B., unpublished).



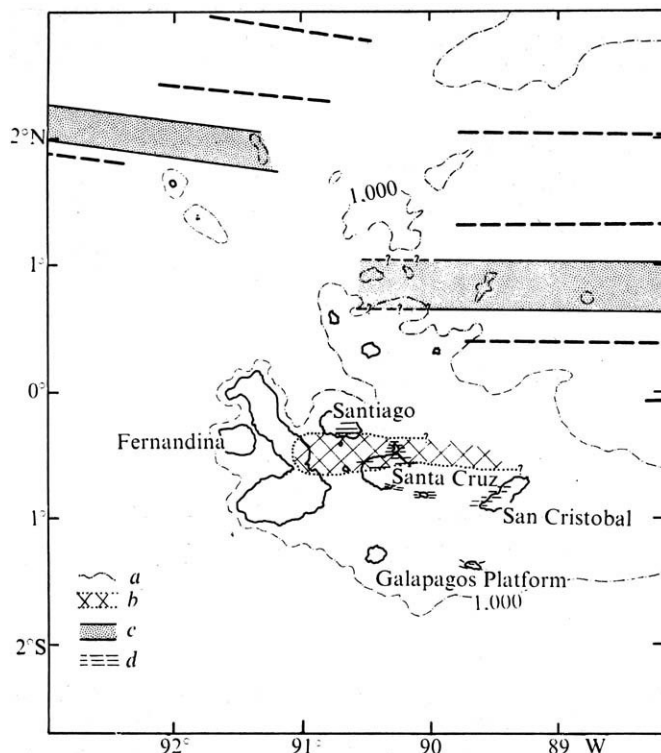


Fig. 2 Galapagos Archipelago. *a*, 1,000 fathom contour; *b*, axis of negative gravity anomaly¹⁸; *c*, Galapagos Rift²⁰; *d*, aligned vents, normal faults, and fissures¹⁷.

by ocean rise-type processes in a fashion similar to the splitting of the ancestral Carnegie Ridge (see ref. 21).

The recent petrological-tectonic history of the central Galapagos could also be interpreted using the currently popular model of mantle plumes. Using a mantle plume hypothesis analogous to the model proposed for Iceland and the Reykjanes Ridge by Sigvaldason *et al.*⁴, the ocean rise-type lavas could be derived from source material progressively depleted during lateral plume flow eastwards from a hot spot at present centred beneath Fernandina Volcano. Other models would derive both low-K₂O lavas and typical oceanic island basalts from different regions within the column of the upwelling plume⁵.

We feel, however, that 'hot spot' and mantle plume models of island development contribute little to our understanding of the history of the Galapagos Archipelago. Clearly, the available geological and geochemical data are insufficient to devise a realistic, unambiguous petrological-tectonic model for the Galapagos region. As in the case of Iceland²² the Galapagos Islands seem to have had a complex history influenced by several distinct but possibly superimposed petrological and tectonic regimes.

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Geomagnetic excursion from Imuruk Lake, Alaska

UNGLACIATED Arctic lake sediments probably offer the best opportunity for correct stratigraphic correlation and radiocarbon dating of brief geomagnetic events.

We possess a series of lake sediment cores from Imuruk Lake, Seward Peninsula, Alaska, which represent a time interval well beyond the range of radiocarbon dating¹. Pollen analysis of Arctic Alaskan lake sediments can be used to identify glacial and non-glacial episodes with considerable precision² and the pollen record from one Imuruk Lake core suggests that at least one, and perhaps two, complete glacial cycles are included¹. Our suite of cores thus offers an opportunity to study the history of palaeomagnetic events over a time interval of perhaps 200,000 yr. We report here the discovery of the Imuruk geomagnetic excursion some 18,000 yr b.p.

Fig. 1 Sampling intervals and geomagnetic excursion interval in Imuruk Lake core IV. N, Normal polarity samples; E, geomagnetic excursion samples. Radiocarbon dates as in Table 1.

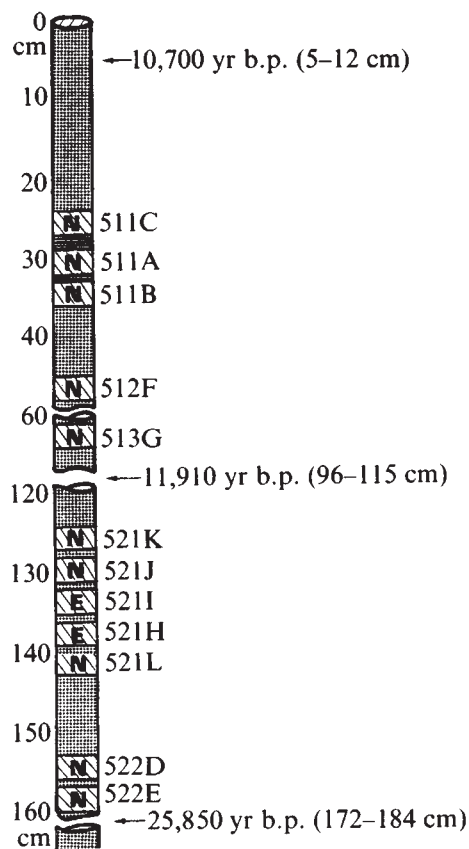


Table 1 Radiocarbon dates on Imuruk Lake core IV

5–12 cm (I-3631)	10,700 ± 150 yr b.p.
96–115 cm (I-3632)	11,910 ± 180 yr b.p.
172–184 cm (I-3662)	25,850 ± 2,400 yr b.p.

The discovery was made with Imuruk Lake Core IV (coordinates 65°37' N, 163°8' W) collected in August, 1960 (ref. 1). The core was stored in the Dural sample tubes with which it was taken until 1967, when it was extruded and examined using radiocarbon dating and pollen analysis³. Since then, the core has been wrapped in Saran film and stored at 4 °C. Core IV is short (2.5 m), but it includes pollen zones, J, K, and L, representing glacial and early postglacial time^{1–3}. Three radiocarbon dates (Table 1) show that the core spans the interval from before 25,000 yr b.p. to about 10,000 yr b.p. This interval spans the estimated ages of a number of geomagnetic excursions, noticeable among them that from Lake Biwa at 18,000 yr b.p. (ref. 4). We found the dating of the Biwa Event particularly interesting because it is synchronous with the last glacial maximum. Accordingly, we chose for our reconnaissance samples the interval of Imuruk core IV in which the sediments of 18,000 yr b.p. must lie.

We removed twelve core samples from the upper 160 cm of Imuruk Core IV (Fig. 1). The cores were measured using a Schonstedt SSM-1A spinner magnetometer. Following the measurement of the natural remanent magnetisation (NRM), the cores were subjected to successive stages of alternating field demagnetisation (a.f.d.) using a Schonstedt GSD-1 demagnetising unit. The total moments of the NRM averaged 5.0×10^{-5} gauss cm³, with individual core volumes of 10 cm³. Ten cores had NRM inclinations averaging 45° and may be considered of normal polarity. Two adjacent cores at 132–135 cm and 136–139 cm (Fig. 1) had NRM inclinations averaging 13°. All of the NRM moments had a similar magnitude suggesting that the NRMs of the two cores with low inclination record a sudden excursion of the geomagnetic field.

We tested the stability of NRM of the samples by demagnetising them in an alternating field to a peak of 400 oersted, in stages of 100 oersted. After a.f.d. to 400 oersted all of the samples showed the same rate of decrease of residual moment to about 25% of their initial values, which suggests that their magnetic mineralogy is uniform (Fig. 2, Table 2). After demagnetisation, the residual inclinations of the samples with normal polarity averaged 48°, a few degrees more than their average NRM inclination. The two samples with low NRM inclinations, however, had residual magnetisations nearly horizontal, 13° less than their average NRM inclination. This indicates that the stable NRM moments in the samples are free of

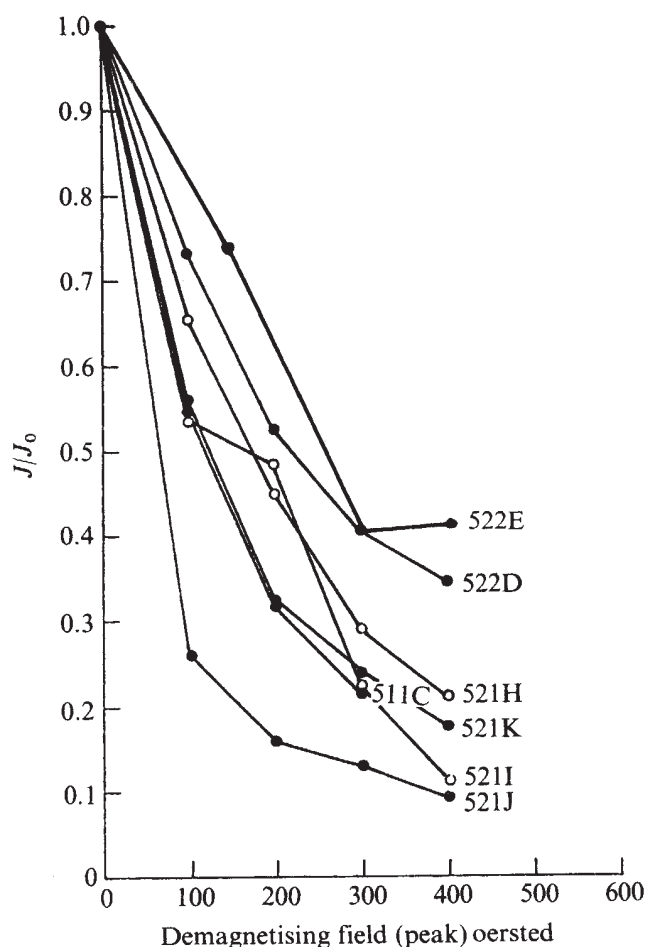


Fig. 2 Alternating field demagnetisation results. ●, Samples with normal (positive) magnetic inclinations; ○, samples with nearly horizontal (excursion) inclinations. J/J_0 is the ratio of NRM to the residual moment after a.f.d.

a consistent bedding plane error, and that there was a change of geomagnetic inclination of approximately 50°. The magnitude of this change is close to the 55° change recorded by Yaskawa *et al.*⁴ from the Lake Biwa (35°13' N, 136°01' E) results. This change was sufficiently abrupt to have occurred between adjacent samples (Fig. 1), suggesting that the decrease in inclination was very rapid, a circumstance which may be tested by investigating the rate at which the Imuruk lake sediments accumulated. It is most unlikely that any of the core samples acquired their NRM long after initial deposition. (Verosub⁵ reports

Table 2 NRM (a) and 400-oersted (b) residual magnetisation results* for seven samples of Imuruk Lake core IV

	Declination (degrees)	Inclination (degrees)	Specimen	Total moment (e.m.u.)
a	206.68	53.22	I4 511C	9.02×10^{-6}
	204.09	47.53	I4 522D	6.17×10^{-5}
	204.30	50.73	I4 522E	3.36×10^{-5}
	171.50	9.12	I4 521I	1.39×10^{-4}
	188.61	17.28	I4 521H	8.92×10^{-5}
	178.07	45.09	I4 521J	5.48×10^{-5}
	199.17	42.11	I4 521K	9.13×10^{-5}
b	131.55	70.45	I4 511C	2.02×10^{-6}
	214.95	53.25	I4 522D	2.12×10^{-5}
	175.67	45.21	I4 522E	1.41×10^{-5}
	173.85	-0.16	I4 521H	1.84×10^{-5}
	184.98	0.49	I4 521I	1.47×10^{-5}
	169.24	35.65	I4 521J	5.17×10^{-6}
	203.18	58.14	I4 521K	1.60×10^{-5}

*The results are plotted in Figure 2. The coring tube was not oriented for azimuth and the declinations are not correct in geographic coordinates.

finding stable NRM in lake sediments acquired within 5 years of deposition.) The conditions in which sediment collects at Imuruk Lake¹ preclude the possibility of small scale folding of the deposit similar to that reported by Verosub⁷ for a glacial lake in Massachusetts which locally reproduced the effect of a geomagnetic excursion. Furthermore, the part of the Seward Peninsula containing Imuruk Lake was free of ice during the Wisconsin glaciation⁹. We conclude that the samples record the orientation of the local geomagnetic field at the time of deposition, and that the low inclination of the two cores represents a real geomagnetic excursion. We have not yet identified the magnetic minerals in the core, but the data are consistent with detrital magnetite contributing about 50% of the NRM, as has been noted in other Recent lake sediments⁶⁻⁸.

The average inclination of the stable NRM moments (48°) in the majority of the samples is about 27° less than that expected at the latitude of 65°N , assuming a geocentric dipole source for the main geomagnetic field. We can reduce the discrepancy to 22° because of a 5° tilt of the lake bed to the south about 10,000 yr b.p.⁹. The inclination of the stable NRM of the majority of the Lake Biwa samples⁴ during the equivalent time interval (11,000–24,000 yr b.p.) averages about 48° , 5° less than expected for a geocentric dipole at 35°N . The present-day geomagnetic inclination in the Seward Peninsula region is 75° (ref. 10), and there is no evidence of a localised anomalous vertical intensity of $-11,000\text{ }\gamma$ near Imuruk Lake, which would be required systematically to diminish by 20° the local geomagnetic inclination during a normal polarity epoch. The low value we observe for the stable NRM inclination may be the result of our small sample, or there may have been a low geomagnetic inclination in the North Pacific region during the Wisconsin Glaciation. The presence of a geomagnetic excursion involving a change in inclination of 50° is, however, clear.

If we assume that the accumulation of sediment between the radiocarbon dates bounding the event (Table 1) is linear, the sedimentation rate during the period of the event is about 1 cm every 193 yr. We can identify the approximate position of the polarity event in other cores using pollen stratigraphy, because the event lies nearly in the middle of pollen sub-zone J_3 (refs 1–3). In Imuruk Core I, taken about one mile from Core IV, the middle of J_3 lies at a depth of 90 cm. This lies between the radiocarbon age of $13,250 \pm 700$ yr b.p. at 50–60 cm, and more than 34,500 yr b.p. at 110–120 cm (ref. 7). Assuming a linear sedimentation rate between these ages, the minimum rate would be 7 cm every 300 yr. In Imuruk Core II, taken a mile from cores I and IV, the middle of sub-zone J_3 lies at a depth of 60 cm and is bounded by radiocarbon ages of $12,160 \pm 300$ yr b.p. at 5–10 cm, and more than 28,700 yr b.p. at 100–114 cm. Assuming a linear sedimentation rate gives a minimum rate of about 1 cm every 200 yr.

The interval of time spanning these pairs of dates includes both intervals of maximal glacial advance and of glacial retreat, so that some fluctuations in the rate at which sediments accumulated must be expected. The assumption of linearity in sedimentation rate cannot, therefore, be valid. Nonetheless the sediments enclosing the geomagnetic excursion in Core IV must have accumulated at a rate of 1 cm about every two or three centuries. At the onset and close of the excursion (Fig. 1) there was a change in the geomagnetic inclination of 50° within 1 cm of sediment (a time interval of 200–300 yr). The excursion persisted during the accumulation of 9 cm of sediment, a time interval of 1,800–2,700 yr.

Our closest estimate for the actual radiocarbon age of the excursion can be obtained from Fig. 3. Figure 3a describes the accumulation of sediments in Lake Biwa^{11,12}, and shows an exponentially changing relationship of age

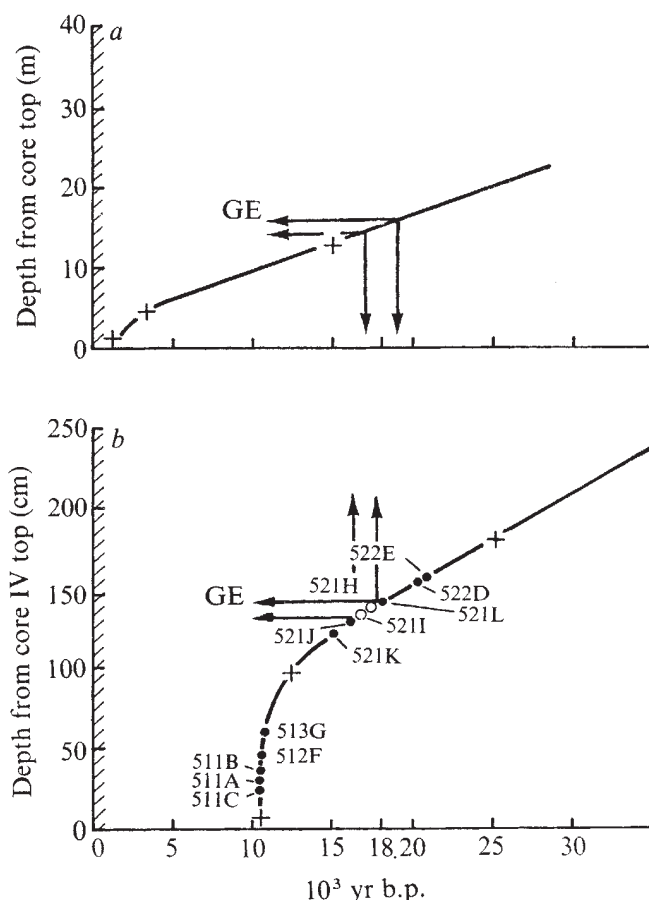


Fig. 3 Sediment accumulation, and geomagnetic excursion interval at a, Lake Biwa⁹ and b, Imuruk Lake. ●, Position of normal polarity samples; ○, geomagnetic excursion samples; crosses, radiocarbon dates; GE, geomagnetic excursion.

against depth as the sediments compact, eventually becoming linear.

Figure 3b is a similar diagram for Imuruk Lake Core IV, based on the radiocarbon dates given in Table 1. The steep age-against-depth relationship in the upper 100 cm of the core mainly reflects the truncation of the lake sediment by wave action in the shallow water of the contemporary lake^{9,13}. The positions of the samples are indicated, and the age of the excursion is between 17,000 and 18,000 yr b.p.

It seems likely that much of the scatter in the ages of polarity events is a consequence of uncertainties in radiocarbon age determinations, or they could arise from linear extrapolation of age when the age-depth relationship is not linear. In the examples of Lakes Biwa and Imuruk, such a linear extrapolation in sediments near the top of the core would result in an underestimate of the age of the polarity event. We speculate that some of the polarity events reported for the 8,000–20,000 yr b.p. interval may be more correctly dated in the 17,000–19,000 yr b.p. interval.

It should be possible to detect the 18,000 yr b.p. geomagnetic excursion (and probably others) in equatorial sediments or igneous rocks where the normal geomagnetic inclinations are close to horizontal, even when cores are not oriented in the horizontal plane. This is because the excursion is accompanied by an apparent shift of the geomagnetic dipole axis by about 30 – 35° , and by an inclination change of 50 – 55° , which is somewhat different from the changes accompanying a geomagnetic polarity reversal. A geomagnetic reversal involves a symmetrical change of

inclination and declination, the inclination changing sign and the declination changing by 180° . It is not necessary to know the declination of a sample if the inclination change is approximately 50° at any latitude during an excursion.

P. Colbaugh undertook the pollen analyses of Core IV and R. Marino assisted with the sub-sampling of the Cores and the magnetometry. The work was supported by the NSF.

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Crystal structure of green rust formed by corrosion of cast iron

THE principal solid corrosion products of iron in water are magnetite and hydrated forms of ferric oxide, depending on the degree of oxidation. The occurrence of intermediate products, termed green rusts, was reported by Keller¹ in 1948, since when the formation and decomposition of these compounds, precipitated from aqueous solution by controlled oxidation of ferrous hydroxide, have been extensively studied^{2–5}. It has been proposed^{2,3} that specific anions are incorporated in the crystal structure of some green rusts, although no substantial evidence has been found to confirm this. A number of green rusts have been classified crystallographically³ as follows: first, rhombohedral green rusts I (GRI) formed in Cl^- , SO_4^{2-} and Br^- solutions and (second), hexagonal green rust II (GRII) formed only in SO_4^{2-} solutions by decomposition of GRI. The interrelationships between the various products involved in the oxidation of ferrous hydroxide have been summarised by Misawa *et al.*⁵ In this paper a preliminary account is given of the crystal structure of a rhombohedral green rust formed by corrosion of iron which, although of

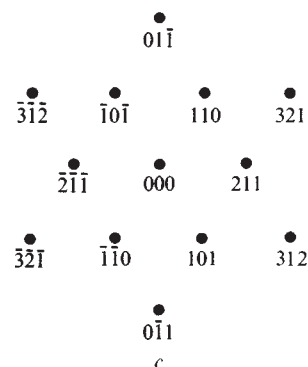
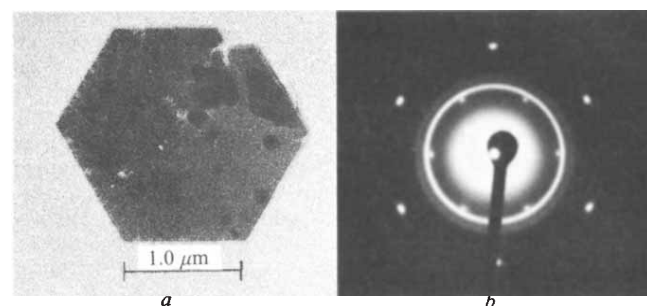


Fig. 1 *a*, Transmission electron micrograph of green rust single crystal. *b*, Corresponding electron diffraction pattern. *c*, Indexed diagram.

similar unit cell dimensions to GRI, differs structurally from those previously reported.

Preliminary corrosion experiments were conducted on cast iron in waters containing NaCl , K_2SO_4 , Na_2CO_3 , mixtures of these salts and NaOH under various degrees of aeration. Optical microscopy showed that the corrosion products contained varying amounts of Fe_3O_4 , $\gamma\text{-FeOOH}$ and a green compound presumed to be a green rust. X-ray powder diffraction confirmed the presence of Fe_3O_4 and $\gamma\text{-FeOOH}$, but

Fig. 2 *a*, Transmission electron diffraction pattern of green rust and magnetite. *b*, Indexed diagram: ○, green rust; ●, Fe_3O_4 . Indices for Fe_3O_4 may be deduced by reference to Fig. 1.

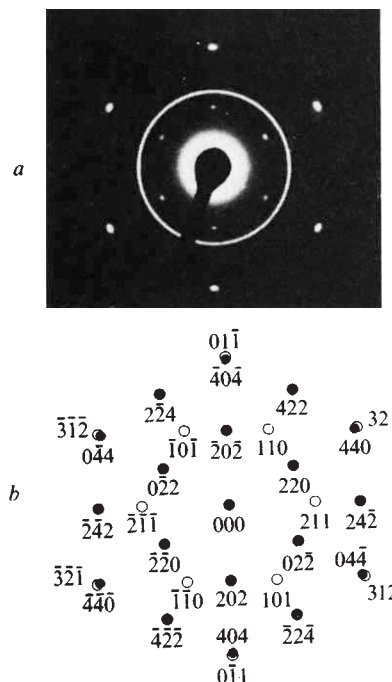


Table 1 X-ray diffraction pattern analysis of green rust on hexagonal and rhombohedral crystal systems

Line number	$d(\text{\AA})$	$(hkl)_H$	$(hkl)_R$	I/I_0
1	7.504	003	1 1 1	100
2	3.755	006	2 2 2	25
3	2.718	101	1 0 0	3
4	2.666	012	1 1 0	20
5	2.462	104	2 1 1	10
6	2.343	015	2 2 1	15
7	2.086	107	3 2 2	2
8	1.964	018	3 3 2	15
9	1.740	10, 10	4 3 3	5
10	1.641	01, 11	4 4 3	3
11	1.584	110	1 0 1	5
12	1.549	113	2 1 0	5
13	1.462	116	3 2 1	5

additional diffraction lines could not be matched with published lines for GRI or GRII. The diffraction pattern for the green compound was found to be independent of the type and combination of anion present in solution and also found to be unaffected by the level of dissolved oxygen. An oxygenated solution of Na_2CO_3 ($\text{CO}_3^{2-} = 200$ p.p.m. by weight) proved to be a convenient medium for growth of the green compound with relatively small quantities of Fe_3O_4 and $\gamma\text{-FeOOH}$ to cause interference. Thirteen unassigned lines obtained in the X-ray powder spectrum for this corrosion scale may be indexed in the rhombohedral system as shown in Table 1. Unit cell dimensions calculated from these data are $a_R = 7.5 \pm 0.26 \text{ \AA}$ and $\alpha = 21.52 \pm 1.15^\circ$; equivalent hexagonal dimensions are $a_0 = 3.181 \pm 0.04 \text{ \AA}$ and $c_0 = 21.82 \pm 0.8 \text{ \AA}$. X-ray diffraction lines, ascribed by Butler and Beynon⁶ to a chloride green rust in studies of corrosion of mild steel in boiling salt solutions, are closely related to those shown in Table 1.

Scanning electron microscopy shows that the green compound comprises clusters of flat crystals with a well-formed hexagonal

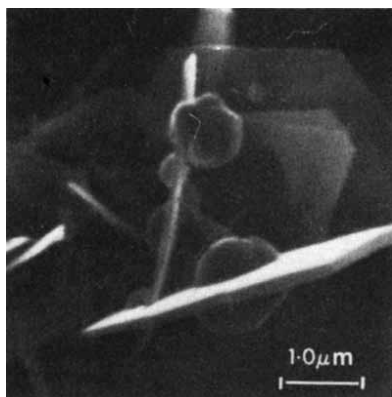


Fig. 3 Scanning electron micrograph showing magnetite growth on hexagonal green rust crystals.

habit. Single crystals were extracted from the corrosion product and prepared for transmission electron microscopy by methods to be described elsewhere. Figure 1a shows a transmission electron micrograph of a single crystal of the green compound and Fig. 1b the corresponding selected area electron diffraction pattern with a superimposed ring pattern from gold used as an internal standard. Figure 1c shows the indexed diffraction pattern. The beam direction is $[1\bar{1}\bar{1}]_R$: two planes of the set $\{211\}_R$ and four planes of the set $\{110\}_R$ are diffracting and have similar d spacings giving the inner group of six spots; the outer group comprises two further reflections from $\{110\}_R$ and four from $\{321\}_R$. The habit plane of the crystal, which is normal to $(\bar{1}\bar{1}\bar{1})_R$ is most closely represented as $\{5\bar{6}\bar{6}\}_R$, and is $5^\circ 48'$ from the hexagonal basal plane, $(0001) \equiv (111)_R$.

Diffraction from certain crystals of the green compound shows a second pattern superimposed on the green rust pattern (Fig. 2a). This can be indexed (Fig. 2b) as magnetite, evidently growing on the surface of the green compound. The orientation relationship between the green compound and magnetite is $[1\bar{1}\bar{1}]_R || [1\bar{1}\bar{1}]_C$ and $(5\bar{6}\bar{6})_R || (1\bar{1}\bar{1})_C$. Morphological evidence for an epitaxial growth of magnetite on the green compound is shown in Fig. 3, in which magnetite crystallites occur on opposite faces of the hexagonal crystals.

It is concluded that the green compound formed by corrosion of iron differs structurally from green rusts I and II produced by solution precipitation. The compound is remarkably stable, and has been found coexisting with Fe_3O_4 and $\gamma\text{-FeOOH}$ in highly oxidising conditions. It is believed that the compound has a significant role in the formation of conventional scales on ferrous surfaces in aqueous environments. Work is in hand on the chemical analysis of the compound.

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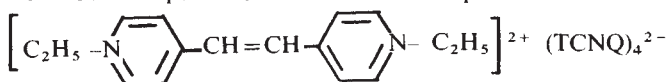
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Organic complex radical anion salt metallic down to 30 mK

THE classical, but still recent, example of an organic metal, TTF-TCNQ, has been fully investigated and reviewed by two groups^{1,2}. It has a room temperature electrical conductivity of $\sim 10^3 \Omega^{-1} \text{ cm}^{-1}$ which increases on cooling to a maximum, with $\sigma_m/\sigma_{RT} \sim 10-15$, at $\sim 58 \text{ K}$ (ref. 1), where it undergoes a metal-semiconductor transition. In 5 of 150 crystals, $\sigma_m/\sigma_{RT} > 20-150$ has been reported¹. Recently³ a congener HMTSF-TCNQ (hexamethylene tetraselenafulvalinium tetracyanoquinodimethane) was reported to have $\sigma_{RT} = 1,391-2,178 \Omega^{-1} \text{ cm}^{-1}$ increasing to $\sigma_m/\sigma_{RT} \sim 3.4$ at 75-45 K, when it decreases to $\sigma/\sigma_{RT} = 0.3$ at 1.5 K. These organic substances showed no superconducting transition, in contrast with the inorganic polymer (SN)_x which, while mostly behaving similarly⁴, also shows the onset of superconductivity at 0.26 K (ref. 5).

Here we report a new organic metal, the first organic complex ever to show a monotonic increase in conductivity on cooling from room temperature to 30 mK. The complex is



1,2-di(N-ethyl-4-pyridinium)ethylene²⁺ (7,7,8,8-tetracyanoquinodimethane)₄²⁻ which we abbreviate to (DEPE)²⁺(TCNQ)₄²⁻.

The complex, prepared in a similar manner to its semiconducting congeners⁶, was grown as single crystals from acetonitrile, and the stoichiometry determined by microanalysis and spectral examination in the visible region⁷. Semiconducting and metallic forms of (DEPE)²⁺(TCNQ)₄²⁻, each with the same stoichiometry, were obtained. The conditions of preparation still remain to be determined completely.

Current and voltage probes were, attached to opposite faces of the crystals, as shown in Fig. 1, with Acheson high conductivity silver paint as electrodes. A constant current was applied and the voltage drop measured by using a Keithley 640 electrometer. The electrode resistance was insignificantly small compared with the sample resistance throughout the temperature range studied.

In this communication we report the preliminary d.c. conductivity data on nine metallic single crystals (mean dimensions $4 \times 2 \times 0.5 \text{ mm}$) isolated from two batches. The conductivities at 300 K of (DEPE)²⁺(TCNQ)₄²⁻, 150-2,200 $\Omega^{-1} \text{ cm}^{-1}$, are among the highest known for any organic material. They may be compared with the value of $\sim 0.26 \Omega^{-1} \text{ cm}^{-1}$ reported⁸ for polycrystalline 1,2-di(N-methyl-4-pyridinium)ethylene²⁺(TCNQ)₃²⁻, a closely related complex. The conductivity increases monotonically on cooling from 340 K to 30 mK (Fig. 1) with $\sigma_{30\text{mK}}/\sigma_{RT} = 3.7$. In the range 50-340 K, $\sigma/\sigma_{RT} = 23T^{-0.55}$ with a weaker dependence at lower temperatures. In this region, a transition to superconductivity was not observed.

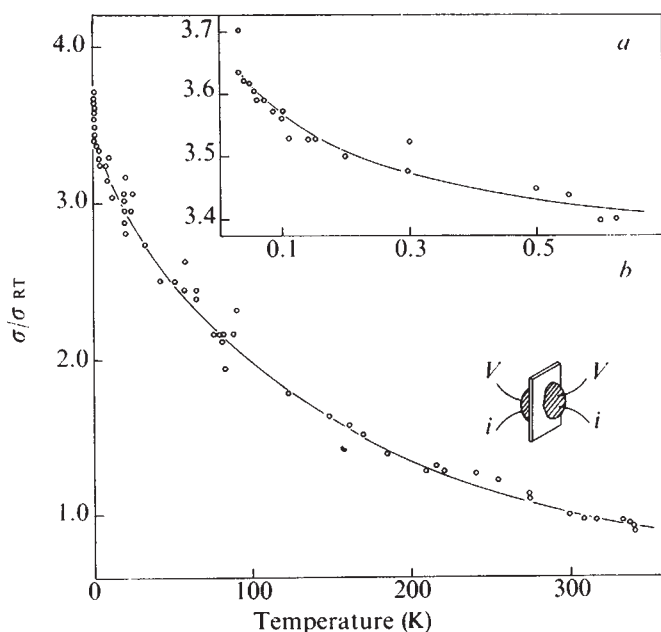


Fig. 1 The normalised electrical conductivity of $(\text{DEPE})_2^{2+}-(\text{TCNQ})_4^{2-}$ as a function of temperature. Insets: *a*, the low temperature region expanded and *b*, the crystal with current and voltage probes attached.

The temperature exponent of -0.55 is considerably less than the values of -2.33 for $\text{TTF-TCNQ}^{9,3}$, and -2.39 for HMTSF-TCNQ^3 . Allender *et al.*¹⁰ have analysed the Fröhlich fluctuation-induced charge density wave conductivity σ_F of a one-dimensional metal obtaining the result $\sigma_F \propto [T_c/(T - T_c)]^{0.5}$, which, while it does not fit TTF-TCNQ , would fit the present data if $T_c \ll T$. If this theory holds (see also Strässler and Toombs¹¹), we shall need to understand why the electron-acoustic phonon interactions would be dominant for our material, while optical phonon interactions must be involved for TTF-TCNQ^{12} . It is also interesting that the σ/σ_{RT} against T plot for $(\text{DEPE})_2^{2+}-(\text{TCNQ})_4^{2-}$ down to 100 K closely follows that for neodymium, where the conductivity is dominated by spin disorder scattering¹³.

The anisotropy of conductivity has still to be established. If it turned out to be two-dimensional, as is found in a related semiconductor⁶, rather than one-dimensional, this might explain the absence of a Peierl's transition. The difference between the metallic and semiconducting species may depend on crystal structure, defect state, or indeed on differences in degree of charge transfer in the surface^{14,15} on which we are now working.

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Model reaction for biological reduction of nitrate involving Mo(III)/Mo(V)

We have characterised the reduction of nitrate by a monomolecular molybdenum (III) species in aqueous solutions. Nitrate reduction is catalysed by nitrate reductases which are molybdoproteins¹. Nitrate is reduced to nitrite by nitrate reductase, the nitrite being further reduced to ammonium ions by nitrite reductase which contains iron but no molybdenum². Evidence indicates that nitrate reductases are monomolecular in molybdenum^{3,4}. Therefore, a molybdenum-based model system for nitrate reductase should be monomolecular in molybdenum and undergo a change of oxidation state of two [$\text{Mo(III)} \rightarrow \text{Mo(V)}$ or $\text{Mo(IV)} \rightarrow \text{Mo(VI)}$].

Advances in the understanding of the chemistry of Mo(III) , (refs 5–8), the observation that Mo(III) reduces nitrate non-enzymatically⁹ and the suggestions^{10,11} that the Mo(III)/Mo(V) couple may be involved in certain enzymes containing molybdenum have led us to study the reaction between the hexaquo-molybdenum cation, $\text{Mo(H}_2\text{O)}_6^{3+}$, and nitrate.

The reaction between $\text{Mo(H}_2\text{O)}_6^{3+}$ and nitrate was followed spectrophotometrically in 1 M *p*-toluene sulphonic acid (HPTS) in strict anaerobic conditions (argon). Representative spectra for both $\text{Mo(H}_2\text{O)}_6^{3+}$ and nitrate are presented in Fig. 1. The absorption spectrum of nitrite in 1 M HPTS exhibits a multi-component band (vibrational fine structure) between 350 and 400 nm which is attributable to the ${}^1B_1 \leftarrow {}^1A_1$ electronic transition ($n \rightarrow \pi^*$). The $\text{Mo(H}_2\text{O)}_6^{3+}$ in the same solvent had a low absorption at 293 nm, indicative of the purity of the preparation⁸. When solutions of $\text{Mo(H}_2\text{O)}_6^{3+}$ ($4.2\text{--}4.7 \times 10^{-4}\text{ M}$) were mixed with excess nitrate ($1.33 \times 10^{-2}\text{ M}$) in pseudo first-order conditions and the reaction allowed to proceed to completion, nitrite absorption between 350 and 400 nm was concomitant with an increase in absorbance at 293 nm. The molybdenum species resulting from the oxidation by nitrate, or by air oxidation in independent experiments, had a spectrum between 340 and 280 nm identical to that of $\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6^{2+}$, as reported by Ardon and Pernick¹².

The kinetics of the reaction between $\text{Mo(H}_2\text{O)}_6^{3+}$ and nitrate were determined using five different preparations of Mo(III) (containing varying amounts of $\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6^{2+}$) at $28.5 \pm 0.5^\circ\text{C}$ in 1 M HPTS in the presence of $1.33 \times 10^{-2}\text{ M}$ nitrate. The $k_f[\text{Mo}_2\text{O}_4^{2+}]$ was found to be $2.92 (\pm 0.27) \times 10^{-2}\text{ l mol}^{-1}\text{ s}^{-1}$. Detectable nitrite was not produced from nitrate in identical conditions when $\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6^{2+}$ —prepared either by the oxidation in air of $\text{Mo(H}_2\text{O)}_6^{3+}$ or by the reduction of Na_2MoO_4 by hydrazine—was substituted for Mo(III) . Moreover, the spectrum of $\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6^{2+}$ in 1 M HPTS did not change during 1 h of observation either in the presence or absence of $1.33 \times 10^{-2}\text{ M}$ nitrate. The reduction of nitrate to nitrite by Mo(V) in the presence of Cl^- in tartrate buffer¹³ is approximately 10 times slower than the reduction of nitrate by Mo(III) reported here.

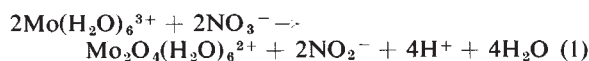
The concentration of the products of the reaction between $\text{Mo(H}_2\text{O)}_6^{3+}$ and NO_3^- indicate the presence of one dimolybdenum species and 2 mol of nitrite (Table 1). The concentration of $\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6^{2+}$ was measured spectrophotometrically, just before oxidation in air, and the concentration of the nitrite was subsequently determined colorimetrically¹⁴. Nitrite eventually disappeared from the solutions, suggesting that Mo(V) reduces nitrite as previously observed¹⁵ in different conditions. When the reaction was allowed to go to completion, all of the $\text{Mo(H}_2\text{O)}_6^{3+}$ present (the total molybdenum)

Table 1 Stoichiometry of the oxidation of $\text{Mo}(\text{H}_2\text{O})_6^{3+}$ by nitrate

Experiment	Total Mo ($\times 10^4 \text{M}$)	$[\text{NO}_2^-]$ ($\times 10^4 \text{M}$)	$[\text{Mo}_2\text{O}_4^{2+}]$ ($\times 10^4 \text{M}$)	Ratio $[\text{NO}_2^-]/[\text{Mo}_2\text{O}_4^{2+}]$
1	4.70	4.90	2.24	2.19
2	4.18	2.37	1.16	2.04
3	4.45	3.24	2.18	1.49
4	4.50	1.28	0.72	1.78
5	4.50	2.44	1.32	1.70

Nitrite measured colorimetrically, $\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6^{2+}$ measured spectrophotometrically. Average ratio and standard deviation of $[\text{NO}_2^-]/[\text{Mo}_2\text{O}_4^{2+}]$ was 1.84 ± 0.28 .

was accounted for in the $\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6^{2+}$ formed. So the overall reaction can be represented by:

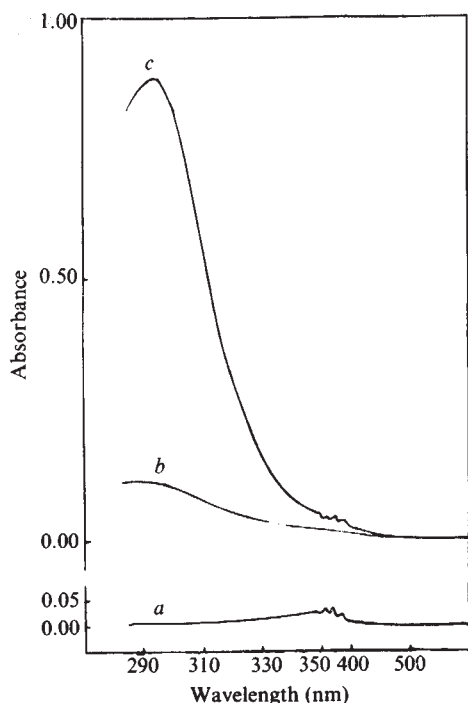


The stoichiometry was confirmed by the values obtained for the ratio $k_f[\text{NO}_2^-]/k_f[\text{Mo}_2\text{O}_4^{2+}]$ (not shown). The nitrite concentration was measured during the reaction by adding sulphanic acid and N-1-naphthyl-ethylenediamine to the reaction mixture and recording the change in absorbance at 560 nm with time. The value of $k_f[\text{NO}_2^-]$ (five independent determinations) was 2.08 ± 0.10 times that of $k_f[\text{Mo}_2\text{O}_4^{2+}]$. The values for the $k_f[\text{Mo}_2\text{O}_4^{2+}]$ were higher in the presence of the nitrite-trapping reagents, which may be attributable to their ability to pull the reaction to completion, and/or the effective removal of nitrite may prevent the further oxidation of $\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6^{2+}$ produced in the reaction.

The reaction of nitrate with $\text{Mo}(\text{H}_2\text{O})_6^{3+}$ is approximately 10 times faster than the reaction reported by Guymon and Spence¹³ for the reduction of nitrate by Mo(V). Moreover, the reaction of K_3MoCl_6 with nitrate at pH 7.0 was too fast to measure by classical techniques, indicating that the reaction between Mo(III) and nitrate is faster at biological pH values than in the acid solutions investigated. We were unable to obtain kinetic data on the reaction between $\text{Mo}(\text{H}_2\text{O})_6^{3+}$ and nitrate at biological pH values because of the formation of molybdenum precipitates.

Nitrate reductases seem to contain one atom of molybdenum at each active centre. Preliminary EPR data demonstrate that

Fig. 1 Spectra of a, NO_2^- ; b, $\text{Mo}(\text{H}_2\text{O})_6^{3+}$; c, $\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6^{2+}$ and NO_2^- produced by the oxidation of $\text{Mo}(\text{H}_2\text{O})_6^{3+}$ by NO_3^- . All spectra in 1 M HPTS.



molybdenum can exist in the (V) oxidation state; one report¹⁶ indicates that the Mo(V) state is the oxidised form of the enzyme, since the EPR signal disappears on reduction of the enzyme. Physiological substrates for nitrate reductases include H_2 , NADPH, H^+ and NADH, H^+ (ref. 1). Therefore, a monomolecular molybdenum species in nitrate reductase undergoes a two-electron redox reaction and it is possible that Mo(V) is the oxidised molybdenum species.

A number of inorganic models for nitrate reductase involving the Mo(V)/Mo(VI) couple have been reported^{13,17-20}. They all involve a single electron transfer and have the disadvantage that nitric oxide or a nitrogen (IV) oxide is formed as the reduced product. They represent models for enzymic nitrate reduction only if one postulates that molybdenum exists in a hydrophobic region of the enzyme. The nitrogen (IV) oxide formed in this hydrophobic region would undergo a disproportionation in the aqueous enzyme solvent resulting in the formation of nitrite and nitrate¹⁸.

On the other hand, the Mo(III)/Mo(V) model shown here involves an overall two electron transfer, could take place in either an aqueous or a hydrophobic (depending on the ligands involved in the molybdenum coordination) region of the enzyme, and produces nitrite as the reduced product and Mo(V) as the oxidised product. Moreover, the Mo(III)/Mo(V) couple is monomolecular in molybdenum; the dioxymolybdenum (V) species being formed in aqueous environments by the dimerisation of a presumed $\text{MoO}_2^+(\text{V})$ species. These arguments suggest that the reduction of nitrate by $\text{Mo}(\text{H}_2\text{O})_6^{3+}$ is a valid model system for nitrate reductase.

Williams²⁰ has postulated an oxygen atom transfer mechanism to explain the biological reduction of nitrate which is reformulated in Fig. 2.

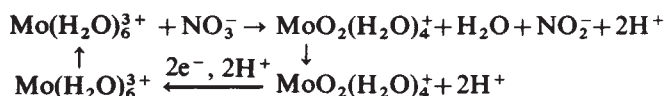


Fig. 2 Postulated oxygen atom transfer mechanism for the enzymic reduction of nitrate by $\text{Mo}(\text{H}_2\text{O})_6^{3+}$, assuming NADPH, H^+ to be the substrate for the reduction of $\text{MoO}_2(\text{H}_2\text{O})_4^+$ to $\text{Mo}(\text{H}_2\text{O})_6^{3+}$. The net overall reaction is $\text{NADPH}, \text{H}^+ + \text{NO}_3^- \rightarrow \text{NADP}^+ + \text{NO}_2^- + \text{H}_2\text{O}$.

This mechanism is consistent with (although not proven by) our data. This scheme postulates that the potential of the enzymic Mo(III) is sufficiently negative to reduce nitrate. The $\text{MoO}_2^+(\text{V})$ produced is reduced by the enzymic electron transport chain which donates 2 electrons and 2 protons. The reactions presented are compatible with the electron transport studies^{16,21,22} on nitrate reductases, which position molybdenum at the terminal end of the electron transport chain and are consistent with the known physiological substrates which donate 2 electrons and 2 protons to the electron transport chain.

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Relevance of oxygen ligands to reduction of ligating dinitrogen

WE have reported¹ the reduction of dinitrogen, in *cis*-[M(N₂)₂-(PMe₂Ph)₄] (*A*, where M = Mo or W), to ammonia, on treatment with sulphuric acid in methanol at 20 °C. The reaction of the molybdenum complex is complete in 3–4 min but the yield (≤ 0.8 mol per Mo atom) is only about half that from the tungsten complex (≤ 1.8 mol per W atom), which requires about 0.5 h for complete reaction. We now find that ammonia is produced almost equally well, (1.7 mol per W atom), but much more slowly, from a methanol solution of *A*, (with M = W) either at the boil (3–4 h) or on irradiation for 30 h at 20 °C (2 × 150-W tungsten filament lamps); a boiling ethanol solution yields only 0.4 mol NH₃ per W atom. In contrast, *A*, (with M = Mo) gives very little ammonia (only 0.08 mol per Mo at best) in any of the above conditions; instead it evolves dinitrogen and dihydrogen.

It is not clear why the molybdenum complex produces so little ammonia from its reaction with methanol alone, in contrast to both its reaction in presence of sulphuric acid and the reactions of its tungsten analogue. One dinitrogen molecule is rapidly lost in all reactions of *A*, (M = W or Mo) leading to some intermediate which, in accord with the general chemistry of molybdenum and tungsten, should probably be more labile in the molybdenum series.

This greater lability may account for a more ready loss of the second dinitrogen ligand from molybdenum and the generally lower yields of ammonia. The greater lability may also be particularly damaging when the intermediate is a methanol- or methoxo-hydrido complex in boiling methanol rather than a sulphato- or other oxo-anion complex at 20 °C. It is, however, possible that the tungsten and molybdenum complexes yield ammonia by different chemical mechanisms².

In both the tungsten and the molybdenum series of complexes, the protonation of N₂ to NH₃ in good yield seems to depend on the replacement of the tertiary phosphine ligands by ligands ligating through oxygen (for example, OH⁻, SO₄²⁻, MeOH). All are replaced from the tungsten complexes, but perhaps only two from the molybdenum. Sulphate is the most effective oxo-anion tried, but others, including phosphate, are almost as good. Chloride and bromide are less effective (our unpublished results).

The importance of ligating oxygen probably lies in its effectiveness as a π -electron donor to early transition metals especially in higher oxidation states. The progressive replacement of the first dinitrogen molecule, which is a strong π -acceptor, and the tertiary phosphine ligands (weak or neutral π -acceptors) by the π -donor oxygen ligands progressively raises the d-electron energy levels on the metal atom, so that electrons pass from the metal to the second dinitrogen molecule as its protonation proceeds; possibly by a mechanism outlined in ref. 2. Chloride and bromide are less effective as π donors and

so lend themselves to the isolation of complexes containing dinitrogen in intermediate stages of reduction².

Oxygen ligands are undoubtedly important in promoting the protonation and reduction of ligating dinitrogen to hydrazine or ammonia in suitable early transition metal (groups IV to VI) complexes. Whenever reasonable yields (for example, > 0.1 mol N₂H₄ or > 0.2 mol NH₃ per transition metal atom) of nitrogen hydrides have been obtained by protonation of a dinitrogen complex, an oxygen ligand, or an oxygen-containing solvent, such as hydroxide³, catechol³, ether⁴, methanol-sulphuric acid¹, propylene carbonate or *N*-methylpyrrolidone⁵, has been involved.

This observation raises the question as to whether, in nitrogenase, the molybdenum, assumed to be the active site, is in an oxygen environment. Chemical evidence seems to point in that direction because almost quantitative yields of hydrazine have been obtained from dinitrogen by its reaction with V(OH)₂ either in aqueous alkaline suspension or in solution with catechol³. This demonstrates that molecular nitrogen can interact with early transition metal ions in oxidation states accessible in aqueous solution, and presumably also in other oxygen or oxygen-nitrogen environments such as may ligate molybdenum in the enzyme.

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Elevation of selenium levels in air by xerography

ROOMS in which xerography machines are used for document reproduction frequently have a strong, characteristic 'selenium' smell. It seemed likely that this is caused by selenium compounds released from the selenium plates or drums charged with high voltages in the photosensitising step of the photoelectric printing process. To test this hypothesis, we sampled the air in an unventilated room (48 m³ in volume) housing a xerographic machine, on which some 450–475 copies were reproduced in intermittent use daily. The air was drawn by a small sampling pump at a rate of 1 foot³ h⁻¹ through a fritted diffuser into 20 ml of 0.1 N NaOH for 9 h d⁻¹ for 4 d. The selenium trapped (0.005–0.015 μ g) was determined according to the fluorimetric method of Olson¹.

Values obtained in replicate runs lay between 20 and 60 ng of selenium (as Se) per m³ of air. The variation in levels may be ascribed to differences in the number of copies made and the non-compliance of users of the machine with a request to keep the door of the room closed during the sampling periods.

The levels found were thus invariably much higher than the 1.2 ng m⁻³ measured in the San Francisco Bay area², a level already considered to be elevated 2,400 times beyond the natural background accountable for in terms of soil and seawater contents. Average levels of 0.9 ng m⁻³ Se were found in seven air samples taken in the Cambridge, Massachusetts area³.

Selenium is an essential trace element for bacteria and animals, and probably also for plants⁴; however, there seems to be a rather narrow range of beneficial selenium availability. Below certain levels, signs of selenium deficiency occur and at somewhat higher levels, signs of

selenium toxicity can become apparent⁵⁻⁷. Tolerable levels are influenced by environmental factors.

Whether elevated selenium levels in xerography rooms are hazardous remains to be seen, but avoidance of selenium buildup by good ventilation may well be indicated.

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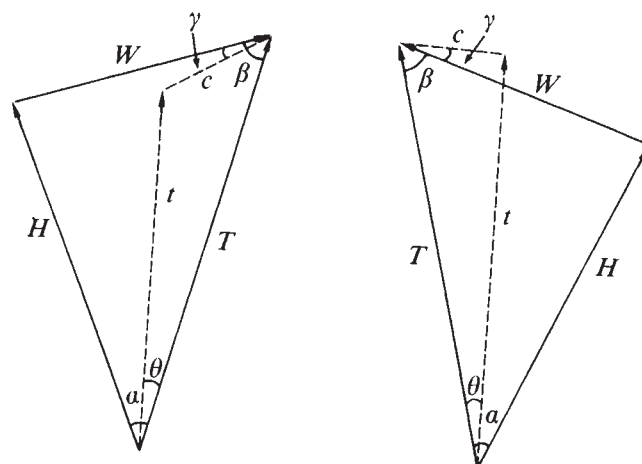


Fig. 1 Vector diagrams of bird movements in relation to wind and sea motion. The birds' ground speed and track direction (T) are available from radar records, geostrophic wind speed and direction (W) from ground synoptic maps. From these data, the birds' heading direction and true air speed (H) can be calculated. If the birds drift because of the wave motion, the direction of velocity relative to the sea (t) should be kept constant irrespective of wind to coincide with the intended track direction, and variation in direction of T should be due to variation in θ . c , Velocity of waves; γ , angular difference between directions of wave movements and geostrophic winds. Dependent on whether winds blow from the left (left diagram) or right (right diagram) of the birds' track direction, γ should be subtracted from or added to β to determine the direction of c . θ could be calculated from the equation:

$$\sin \theta = c \sin(\beta \pm \gamma) [c^2 + T^2 - 2cT \cos(\beta \pm \gamma)]^{-1/2}$$

significantly less over land than over the sea although the same birds were studied in both cases. Thus, pseudodrift cannot explain the increased amount of drift over the sea. Furthermore, these observations are at variance with the second and third (as far as pure inertial navigation is concerned) hypotheses.

In this report we present support for our hypothesis that the drift over sea was due to the birds using the wave pattern as 'landmarks'. It is reasonable to expect that the birds were not aware of the movement of the waves, but experienced the sea as a stationary landscape. We propose that the birds behaved in an analogous way over sea as over land, that is they projected their intended flight direction visually on to the wave pattern and headed into the wind to be able to fly along this course. The birds may determine the angle between their track direction and the waves by referring to irregularities, such as smaller or larger waves, of the wave landscape. (An inertial-Doppler navigating system would also be compatible with our hypothesis²⁰.) Thus, according to our hypothesis, the birds compensated completely for wind drift in relation to the system of waves, and the resulting drift, as observed on the radar screen, was due to the motion of the sea relative to land. Orientation with reference to ocean waves has been suggested before^{18,24}, but the consequence of drift relative to land has not been considered. This hypothesis may give a complete or partial (in combination with pseudodrift) explanation for drift over the sea, as witnessed in several studies^{8,10,11,13,16}.

To test this hypothesis we used our data on crane, wood pigeon and redwing migration to calculate the angle between the

Do birds use waves for orientation when migrating across the sea?

MIGRATING birds apparently can determine their intended flight direction with reference to the Sun, the stars or the magnetic field¹⁻³. This mechanism, however, cannot be used to prevent wind drift. We suggest that for this purpose they orientate themselves with reference to the waves when flying over the sea.

Low-flying, diurnal migrants have been found to compensate completely for wind drift over land^{4,5}. Most radar studies have generally produced evidence of at least partial compensation in both nocturnal and diurnal migrants over sea as well as over land⁶⁻¹³, although some investigations indicate uncompensated wind drift¹⁴⁻¹⁷. Partial compensation has been interpreted in terms of 'pseudodrift', that is complete compensation is thought to take place, but populations with different intended track directions migrate in different proportions in different wind conditions^{8,10}.

The following means to achieve complete compensation have been proposed¹⁸: (1) continuous reference of the flight track to the landscape^{4,7}, (2) reference to the wind in combination with initial or occasional reference to landscape^{9,19}, (3) inertial recording of rectilinear and angular accelerations^{7,20}.

Radar studies in southern Scandinavia of the diurnal migration of cranes *Grus grus*²¹, wood pigeons *Columba palumbus*²² and redwings *Turdus iliacus*²³ have revealed incomplete compensation for wind drift over the sea, but complete compensation over land (no data were available for redwing migration over land). The variation of track directions over the sea was significantly less than would be expected if the birds drifted from fixed heading directions. Pseudodrift was observed for pigeons over land in southernmost Sweden as birds departing from different geographical areas flew in slightly different directions, and the proportion of pigeons arriving from the different areas varied with wind conditions. The drift effect was, however,

Table 1 Predicted amount of drift for birds migrating over the sea and orienting with reference to the wave pattern as compared with drift observed

	Fbo-L	Predicted	Fbo-S	Fln-L	Fln-S	Observed ±95% confidence interval
Crane, Baltic Sea	0.40		0.51	0.29	0.41	0.25±0.15
Wood pigeon, Baltic Sea	0.34		0.48	0.26	0.40	0.36±0.17
Wood pigeon, Kattegatt	0.27		0.32	0.29	0.33	0.26±0.21
Redwing, Baltic Sea	0.08		0.16	0.09	0.17	0.17±0.16

Amount of drift is expressed as linear regression coefficients of θ on α (predictions) or of birds' track directions (T) on α (observations, see Fig. 1). Four predictions have been calculated on the basis of wave measurements at two different sites (Fbo and Fln) and of two different relationships between surface and geostrophic winds (see text).

Table 2 Coefficients of linear regression of birds' track direction on the relative motion of the sea

Predicted		Observed $\pm 95\%$ confidence interval (correlation coefficient)			
		Fbo-L	Fbo-S	Fln-L	Fln-S
Crane, Baltic Sea	1	0.54 ± 0.28 (0.65)	0.48 ± 0.26 (0.64)	0.74 ± 0.39 (0.65)	0.59 ± 0.33 (0.64)
Wood pigeon, Baltic Sea	1	0.98 ± 0.48 (0.67)	0.75 ± 0.35 (0.69)	1.33 ± 0.64 (0.68)	0.93 ± 0.43 (0.69)
Wood pigeon, Kattegatt	1	0.83 ± 0.43 (0.76)	0.67 ± 0.34 (0.76)	1.14 ± 0.59 (0.76)	0.89 ± 0.46 (0.76)
Redwing, Baltic Sea	1	1.87 ± 1.45 (0.70)	0.93 ± 0.71 (0.70)	1.99 ± 1.50 (0.71)	1.04 ± 0.77 (0.71)

Linear regression coefficients of T on θ (Fig. 1) are calculated (compare Fig. 2). Correlation coefficients are in parentheses. If birds fly on constant track directions relative to the sea, regression coefficients would be 1.

birds' track directions in relation to ground and sea, respectively, for each day of migration (Fig. 1) on the basis of the following assumptions.

(1) Waves move parallel to the surface wind direction. This assumption is of course violated in cases of rapid shifts of wind direction, but is a justifiable approximation since tides and currents are of no or little importance in the study areas²⁵.

(2) Wave velocity (c) was estimated from surface wind velocity on the basis of (a) measurements of wave periods in relation to winds at the lightships Falsterbo Rev (Fbo) in the southern Baltic Sea and Fladen (Fln) in the Kattegatt²⁶ (the wave period was calculated as a linear function of surface wind velocity in the interval 3–14 ms^{-1}) and (b) the theoretical relationship between wave period and velocity ($c = p(g/2\pi)$, where p is wave period, g is acceleration of gravity)²⁷. The lightships were situated at sea areas with sufficient depths (30–50 m) for this factor not to impose any restrictions on wave propagation. The difference between wave movements with onshore and offshore winds (the lightships were situated 10–20 km offshore) was very small, and the combined sets of wave data were used. There was, however, a significant difference between wave movements at the two measuring stations, which cannot be explained. We therefore made separate calculations for the two stations.

(3) The birds travelled at altitudes of several hundred to a thousand metres. Direction and speed of the winds were evaluated from barometric pressure charts (geostrophic winds). Because of friction the surface wind force is reduced to about 70% of the geostrophic wind speed over the open sea (S) and 46% over land (L)²⁸. Intermediate conditions would be expected in the study areas, due to the proximity of land, and we made separate calculations for both cases. On the basis of the above assumptions and calculations wave velocity was estimated from geostrophic wind velocity (W) according to the equations:

$$\text{Fbo-L: } c = 3.32 + 0.111W; \quad \text{Fln-L: } c = 1.98 + 0.137W;$$

$$\text{Fbo-S: } c = 3.32 + 0.176W; \quad \text{Fln-S: } c = 1.98 + 0.219W.$$

These equations refer to average conditions determined from several thousands of observations of wave periods, and relationships would be expected to deviate at specific occasions, especially when the wind speed changes rapidly.

(4) Friction causes the surface wind direction to shift anti-clockwise in relation to the geostrophic wind direction. The angle of deviation (γ , Fig. 1) is about 14° over open sea and 38° over land²⁸.

The amount of drift can be estimated on a scale from 0 (birds are flying on constant track directions) to 1 (fixed heading directions) if, by way of linear regression analysis, track directions are related to the angle between track and heading directions^{21–23}. The amount of drift observed over sea varied between 0.17 and 0.36. These figures differ significantly from 0 as well as 1 and therefore lend no support to the hypotheses that the birds compensated completely for wind drift or that they drifted from fixed heading directions^{21–23}. The observed amount of deflection is, however, close to what is predicted if the drift is due to the wave motion (Table 1).

If the birds are deflected due to the motion of the sea, the variation in observed track direction should be due to the variation of the angle between T and t as defined in Fig. 1. This

relationship is examined in Table 2 and Fig. 2.

Considering the uncertainties involved in applying data on the averaged relationship between waves and winds for specific instances, we think that the results form good support for the hypothesis that birds drift because of the motion of the sea.

The crossings over the southern Baltic Sea and the Kattegatt

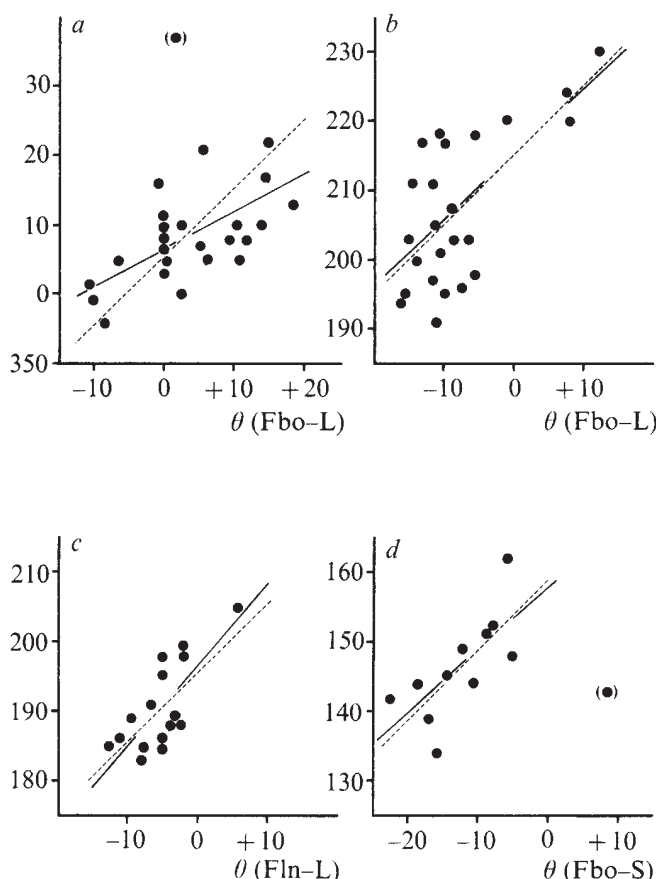


Fig. 2 Flight directions of birds (T) during sea crossings in relation to the angle between track directions relative to ground and sea, respectively. The broken line (regression coefficient = 1) shows the relationship to be expected if the birds fly on fixed track directions in relation to the sea and drift by the motion of the sea. The solid line shows the linear least-squares fit to observational data (compare Fig. 1 and Table 2). One observation of crane migration is bracketed and excluded from the analysis. Mean track direction (037° , only few flocks migrating) was considerably more towards the east than on any other day. Weather was different from all other occasions; strong west-south-west winds (approximately 20 ms^{-1}), low cloud ceiling, bad visibility and rain prevailed during the passage of a cyclone over southern Scandinavia. We suggest that the birds could not detect the sea, but drifted from a heading approximately pointing towards the preferred track direction ($\alpha = 42^\circ$). Furthermore, data from 1 d of redwing migration are excluded, due to uncertainty about prevailing winds²³. a, Crane, Baltic Sea; b, wood pigeon, Baltic Sea; c, wood pigeon, Kattegatt; d, redwing, Baltic Sea.

extend over fairly short distances (50–150 km), but cranes regularly cross much wider areas of open sea, such as the eastern Mediterranean, sometimes by night²⁹, and the redwing, which typically migrates at night, regularly covers still longer distances over the open sea, for example, the North Sea from Norway to Britain and the Atlantic from Iceland to Britain²⁴.

Without any points of reference for the track of flight, the birds may have to resort to heading towards the intended track direction and would consequently be drifted by the wind (or by clouds)³⁰. By using landmarks or wave patterns they can completely (over land) or partly (over sea) compensate for the drift. Navigational ability may furnish birds with the possibility of reaching their goals in spite of drift over sea, by way of continually readjusting the intended track direction towards the goal.

It has been demonstrated that pigeons manage to home when released with frosted contact lenses (preventing detection of landmarks)³¹ or over the sea with no sight of land^{32–34}. In both cases, however, homing performance was worse, that is more pigeons were lost and loft re-entry times were longer, than for control birds released over land. The poor homing performance may be due to drift by winds and waves, respectively.

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Oscillations of tropical insect populations

SAUNDERS AND BAZIN¹ have suggested that “only ecosystems with relatively few species can have a sufficiently high linear connectivity to produce oscillations without becoming unstable, which would explain why it is that population oscillations are observed in the Arctic but not in the

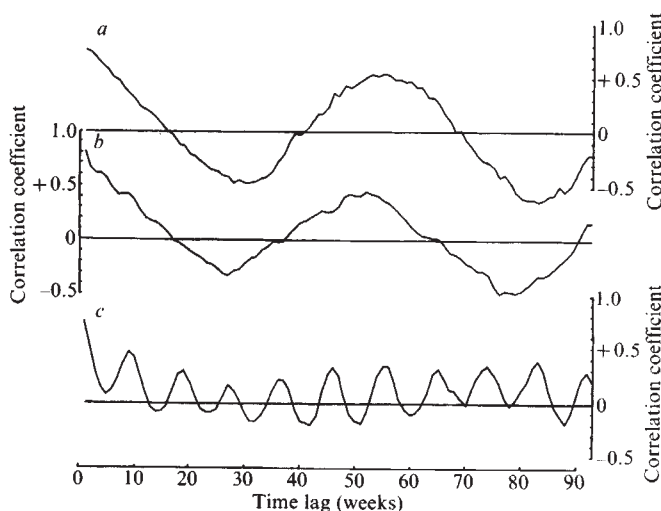
tropics”. Most numerical studies of insect populations in the tropics have been relatively short but what longer time series there are do not bear out this statement. The more favourable temperature conditions in the tropics can allow breeding to continue throughout the year, and typically 8–10 generations may be completed each year.

Nevertheless, annual cycles of abundance are commonplace and, not infrequently, shorter term oscillations with a period equal to the generation length are evident, they must arise through the delay caused by the insect passing through its developmental stages. These ‘generation’ cycles are remarkably persistent and do not damp out with time as predicted by Lotka². They are particularly strong in suction trap records of the coffee leaf-miners, *Leucoptera meyricki* (Guesq.) (Lepidoptera, Lyonetiidae) and *L. coffeina* (Washb.) in Tanzania³, and are observed in populations of *Planococcoides njalensis* (Laing) and *Planococcus citri* (Risso) on cocoa in Ghana. Furthermore, the idea that the greater complexity of tropical ecosystems necessarily confers on them a higher degree of stability and thus that oscillations tend to damp out seems to be largely illusory.

Of all tropical tree crops, cocoa maintains one of the most complex insect ecosystems. On a world-wide basis Entwistle⁴ has recorded more than 2,000 associated insects, and in Ghana some 250 ant species have been recorded from cocoa. The crop is grown under thinned forest shade and is maintained in its early years with the minimum of disturbance to the underbush vegetation which persists until the cocoa canopy becomes sufficiently thick to shade it out. It is thus in intimate contact with an overstorey of forest trees and an understorey of ground vegetation, and receives its insect fauna from both sources. In particular, the ant regimes which make up the bulk of the insect biomass on cocoa seem to be superimposed on the crop from the overstorey trees. The interrelationships between the components of the cocoa ecosystem are very complicated, involving homopteran–ant mutualistic associations and territorial antagonism between the more aggressive ant species, which leads to the formation of discrete, three-dimensional ant mosaics⁵. Agents such as the cocoa mirids and the mealybug-transmitted, cocoa-swollen shoot virus can lead to the large scale degeneration of the cocoa canopy with a consequent impact on the other elements of the ecosystem.

Yet in spite of the complexity of the cocoa ecosystem

Fig. 1 Serial correlations of a time series comprising 186 weekly records of the percentage of trees infested by three different insect species on a block of 238 Amazon cocoa trees. *a*, *Steatococcus* sp.; *b*, *Pheidole megacephala* (F.); *c*, *Phenacoccus hargreavesi* (Laing).



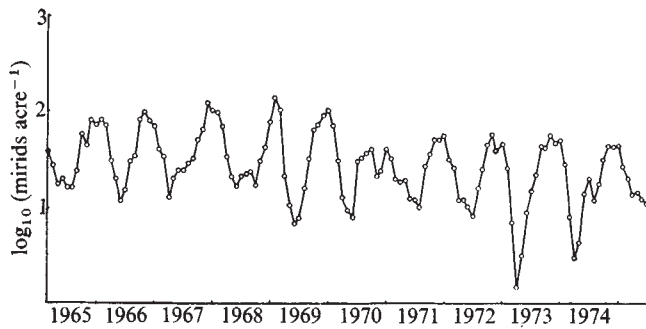


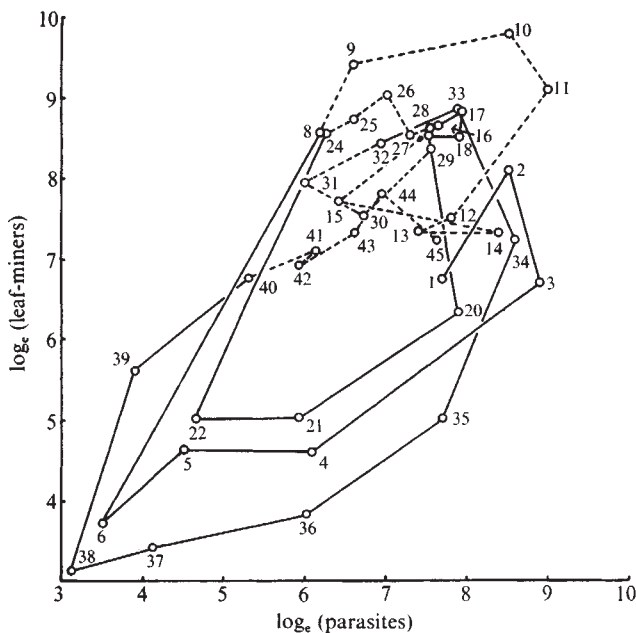
Fig. 2 Monthly assessment on 25 farms in eastern Ghana of populations of the cocoa mirids *Distantiella theobroma* (Dist.) and *Sahlbergella singularis* (Hagl.). The counts of the two species have been combined.

many of its component populations oscillate both numerically and spatially. Figure 1 shows three correlograms obtained from a serial correlation of time series of 186 weekly records of the percentage of trees infested on a block of 238 Amazon cocoa trees. *Steatococcus* sp. (Hemiptera, Coccidae) and *Pheidole megacephala* (F.) (Hemiptera, Formicidae) show particularly strong oscillations with a period of about 1 yr, and *Phenacoccus hargreavesi* (Laing) (Hemiptera, Coccidae) shows a cycle with a period of 9.2 weeks.

Population counts of the cocoa mirids, *Sahlbergella singularis* (Hagl.) and *Distantiella theobroma* (Dist.) made at monthly intervals on 25 farms in eastern Ghana since 1965 (Fig. 2) showed an annual cycle in abundance, and perhaps a much longer term cycle.

In Tanzania, daily catches in suction traps of *Leucoptera meyricki* and *L. coffeina* on arabica coffee were made at Lyamungu, on the southern slopes of Mount Kilimanjaro between 1960 and 1968, covering in all 62 moth generations^{3,6}, and for the last 45 generations independent estimates have been made of the density of the larvae of the two species and their associated parasites (Fig. 3). It is clear that a 16-generation (two-year) cycle is superimposed on an 8-generation (one-year) cycle. A clearer picture of

Fig. 3 Number per generation per 100 leaves of larvae of coffee leaf-miners and their larval parasites on arabica coffee in Tanzania. Generations are numbered consecutively, solid and dashed lines indicating alternate years. No counts were made in generations 7 or 23.



the perturbations of the combined host-parasite system about its mean level can be obtained by a principle components analysis of logarithmically transformed values of hosts and parasites since values along the first components axis are a linear combination of the host and parasite scores (Fig. 4). Here again the two-year cycle is very evident. Population counts of the two leaf-miner species were made at Lyamungu by Notley^{7,8} between 1938 and 1940, and a similar two-year cycle is discernible in his data.

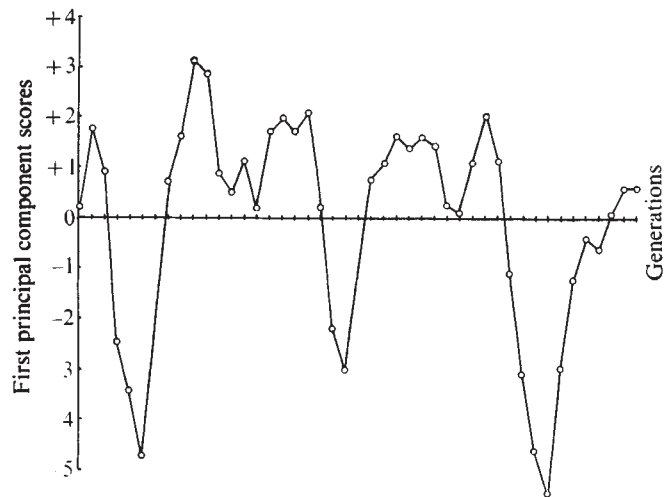


Fig. 4 Displacement from equilibrium of the leaf-miner-parasite system of Fig. 3 considered as a whole and expressed as distances along the first principal component axis from a principal component analysis of the logarithmically transformed numbers of leaf-miners and parasites per generation.

It seems likely that the annual leaf-miner cycle is driven by seasonal weather changes and is not caused by interactions within the system, but it is not at present obvious how an annual weather pattern could interact with the system to produce a two-year cycle. There is some evidence that the leaf-miner parasites do no more than follow the cycles of leaf-miners.

It could be argued that the lynx cycle, to which Saunders and Bazin¹ are presumably alluding in their reference to the Arctic, is not comparable to the leaf-miner cycle because of the great difference in periodic lengths but when the two cycles are compared in terms of generation length this apparent difference disappears. The driving force behind both cycles is obscure. Bulmer⁹, has suggested that the cycles of Arctic predators are caused directly or indirectly by the cycle in the snowshoe rabbit but this leaves unanswered the question of why the snowshoe rabbit population should be cyclical.

Williamson¹⁰ has suggested that the standard deviation of the $\log_{10}$ generation values can be used as a measure of population variability and thus of the amplitude of oscillations, and he quotes a number of examples. The standard deviation of the leaf-miner population is 0.768, which compares with a standard deviation of 0.558 for the lynx cycle. The leaf-miner standard deviation is similar to many of the European insects cited by Williamson¹⁰ and compares with a value of 0.771 observed for the black headed budworm *Acleris variaria* (Fern) and 0.595 for the European spruce sawfly *Diprion hercyniae* (Htg) in Canada, calculated from data given by Morris¹¹.

It is clear that not only do tropical insect populations show cyclical behaviour, but also that the amplitude of the cycles is comparable to those of temperate insects. Furthermore, even in the cocoa ecosystem, which is considerably complex and has many features in common with tropical forests, population cycling is observed in many species.

Tropical insect host-predator systems are worthy of much

closer examination if only because they have the advantage over temperate systems of a compression of the time scale by a factor of 8 to 10.

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Divergent X-ray-induced mutation rates in the mouse for *H* and “7-locus” groups of loci

INCREASED demand for nuclear power re-emphasises the need for a well-grounded estimate of the genetic risk of environmental mutagens in general and of ionising radiations in particular. With respect to germ-line, specific-locus mutation rates, estimates for man have been based primarily on the mouse 7-locus test, carried out on a large scale at Oak Ridge National Laboratory (USA) and Harwell (UK). Among the many important radiobiological findings recently summarised¹ are the following: the mutation rate depends on sex, on the type of germ cell irradiated, on radiation quality (X rays, neutrons), on dose rate (rad min^{-1}), on fractionation (number of treatments during a certain period) and on total dose.

With this information in hand, we can inquire about the limitations inherent in the 7-locus test. First, is the sample of seven loci (*a*, *b*, *c^h*, *p*, *d*, *se*, *s*) representative of the entire mouse genome? Second, does the test, detecting so far only the change from dominant to recessive (it can also detect wild type to dominant), adequately approximate the rates for other types of specific-locus mutational change? Two reports bear directly on these questions.

Lyon and Morris², using 7-locus test methodology, tested five other loci together with one of the original seven (*a*, *bp^h*, *fz*, *ln*, *pa*, *pe^h*) in a “6-locus” test. Dominant-type males were mated to untreated females which were homozygous for recessive alleles at the loci of interest. Mutants were detected in the *F*₁ progeny by their visible recessive phenotypes, that is, by a loss of dominant allele activity. After 600 rad of X rays, the mean spermatogonial mutation rate was one-third that of the 7-locus mean.

Bailey and Kohn³ screened a different and much larger (>30) set of loci (histocompatibility or *H*) whose alleles are codominant. The *H* test detected losses in antigenicity (analogous to the loss of the dominant phenotype in the 7-locus test), and also a change or gain in antigenicity, which might signal a change in one or more codons or the appearance of a new active gene. The result of their experiment with a single dose of 522 rad suggested that the *H* loci were less sensitive than the “7 loci”, but did not establish the size of the difference.

We have therefore made a quantitative comparison. We used a standardised *H*-test procedure^{3–5} in which tail-skin grafts were orthotopically exchanged in a “reciprocal circle” among syngeneic mice, 37–43 d old. Each mouse provided one graft for each of two other mice of similar sex and received two grafts in return. All grafts should have been accepted. Suspected mutants, detected on the basis of

Table 1 Predicted number of mutants per dose group based on the results with the 7-locus test

Dose (rad)	H tested <i>F</i> ₁ s	<i>K</i> ($\times 10^{-7}$)	Predicted nos of induced mutants
Controls	2,335	—	—
Single doses			
350	1,239	2.8	3.6
500	947	2.5	3.6
650	1,285	2.2	5.5
800	1,603	1.65	6.3
Split, 1 d			
500 (150 + 350)	1,107	3.35	5.6
650 (300 + 350)	1,339	3.7	9.7
800 (300 + 500)	1,061	4.1	10.4
Split, 5 or 8 weeks			
650 (300 + 350)	1,422	2.8	7.8
800 (300 + 500)	1,276	2.6	8.0
Total treated	11,279	—	60.5

The predicted number of induced mutants was equal to: *H* loci tested per irradiated gamete (30) $\times$ dose (rad) $\times$ animals tested $\times$ *K*. *K* (expected rate of induction of mutations per locus per rad) was calculated separately for each of our dose groups on the basis of the Oak Ridge and Harwell statistics for the 7-locus test, given in Tables VI and VII of ref. 1. The doses there are in r.; we used them as equivalent to rad. For single exposures, the value of *K* was read from a four-point curve over the range 300–900 r. For split doses 1 d apart, *K* was read from a line determined by two points, that for a single dose of 300 r. and that for 1,000 r. given in two 500 r. fractions. This made the conservative assumption that splitting 300 r. did not increase *K*, but that such split doses become relatively more effective as the total split-dose rose to 1,000 r. For split doses 5 or 8 weeks apart (mean 52 d), no effect of fractionation was assumed; *K* for each fraction was that read from the single-dose curve. The values for the two fractions were combined to give the single equivalent value of the table.

their pattern of graft rejection 57–63 d after grafting, were backcrossed to establish transmission of mutations.

The grafted animals were the *F*₁ progeny of pretested C males (BALB/cKh) whose pelvic region had been X rayed at 105–152 d old, and who were mated 75 d later to pretested B6 females (C57BL/6Kh). The radiation factors were 250 kVcp X rays, half value layer 1.2 mm Cu; intensity, 60–65 rad min^{-1} . We used 34 control and 214 irradiated males, who were mated with 692 B6 females. The males were kept in service for 1–1.5 yr after initiation of mating, and were mated with one female at a time.

Two classes of loci were at risk^{6,7}. Class I loci were heterozygous in the *F*₁ animal because the B6 and C parents carried different alleles. A minimum of 30 such loci was irradiated in the C male and tested for spermatogonial mutations in the BC6.*F*₁ progeny. Class II loci were homozygous and will be dealt with in a later report. Results with class II loci are consistent with those for class I.

In all, 11,279 *F*₁s were tested for the effects of single and split spermatogonial doses of 350–800 rad. There were 2,335 controls. Results with the 7-locus test predicted that the number of induced mutants would be 60 (Table 1). We observed only one class I, C-allele mutant in the treated groups. It was of the “gain and loss” type (a specificity was lost and was replaced by a new one); its father had received 650 rad (300 + 350, 1 d apart). Four other class I mutations were detected (three gain and loss, and one loss), but had occurred in B6 alleles, that is, in the unirradiated maternal gamete. No class I mutants were detected in the controls. Failure to find mutants was not considered to be a failure to detect them, as more than 60 class I and II mutants in 30,000 mice have been reported^{3,6}.

The results for the mouse with the 7-locus, 6-locus and *H* tests show that different specific-locus tests applied to the same germ cell (spermatogonium) yielded different X-ray-induced mutation rates (relative values of 60, 20 and 1, respectively). This raises the question, to what extent can any particular set of loci represent the mammalian genome in this field? The results with the *H* loci, of course, might

indicate unique factors in histocompatibility radiation genetics that do not apply elsewhere. In any event, it appears that further research with various loci is needed.

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Specific marker chromosome 14 in malignant lymphomas

A CONSISTENT structural abnormality of chromosome 14—an extra band at the terminal of the long arm—has been found in 10 out of 12 African Burkitt lymphomas¹. We report here a similar long acrocentric marker chromosome derived from chromosome 14 in other types of malignant lymphoma.

The samples used were seven lymphosarcomas, three reticulum cell sarcomas, six Hodgkin's lymphomas and one follicular lymphoma. The lymphoma cells from lymph nodes, peripheral blood, bone marrow, ascites, pleural effusion and a cell line *in vitro* were cultured for 24 h in RPMI 1640 medium with 10% foetal calf serum, and the chromosome specimens were prepared as usual and banded with Giemsa². As controls, lymph node cells from three cases with chronic lymphadenitis were examined.

Table 1 summarises the results obtained. Many cells from various lymphoma cases reveal a long acrocentric marker chromosome. This marker was found in three out

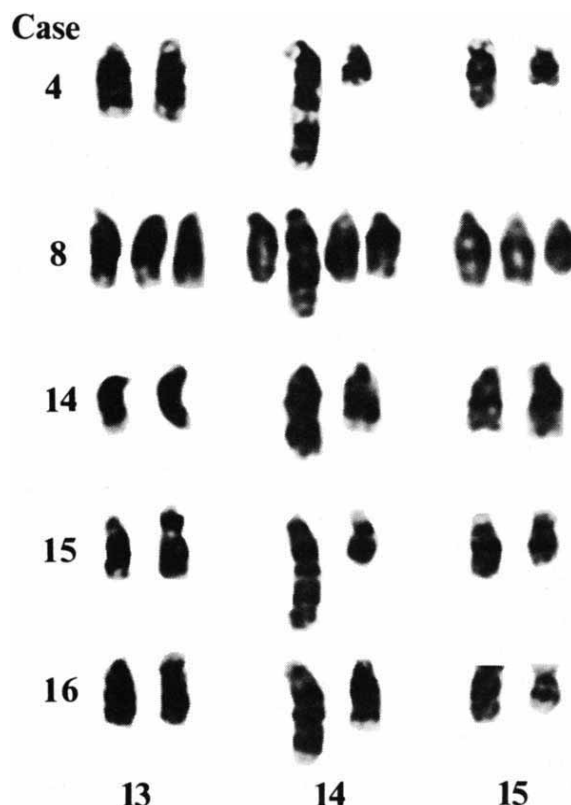


Fig. 1 Partial karyotype of D-group chromosomes of five mitotic malignant lymphoma cells banded with Giemsa. Note a longer chromosome 14 than usual in each case.

of four cases of lymphoblastic lymphosarcoma and absent in three lymphocytic lymphosarcomas. About 50% of the hypotetraploid cells of reticulum cell sarcoma in cases 8 and 9 had a similar marker although in case 10, examined after irradiation, this marker was not frequent. A similar acrocentric marker was found in three out of six cases of Hodgkin's disease. Cells with this marker were hyperdiploid in case 14, and near triploid in case 15. Both hypodiploid and near triploid cells in case 16 had this abnormal chromosome. Cases 11, 12 and 13 which were in a less advanced histological stage lacked this acrocentric marker. Abnormal

Table 1 Incidence of long acrocentric marker chromosome in malignant lymphoma cells

Case no.	Diagnosis	Total cells counted	Incidence (%)	Cells with the marker Total cells counted × 100
1	LSaL (lymphoblastic)	58	0	
2	LSaL (lymphoblastic)	46	19	(65% of hyperdiploid cells)
3	LSaL (lymphoblastic)	31	30	(all hyperdiploid cells)
4	LSaL (lymphoblastic)	50	100	(all near diploid cells)
5	LSaL (lymphocytic)	27	0	
6	LSa (lymphocytic)	28	0	
7	LSa (lymphocytic)	30	0	
8	RCSa	28	54	(60% of hypotetraploid cells)
9	RCSa	40	45	(50% of hypotetraploid cells)
10	RCSa	50	20	(22% of hypotetraploid cells)
11	HD (LP)	68	0	
12	HD (LP)	50	0	
13	HD (MC)	69	0	
14	HD (MC)	50	98	(all hyperdiploid cells)
15	HD (LD)	50	78	(all near triploid cells)
16	*Hodgkin cell line	50	100	(all hypodiploid and near triploid cells)
17	FL	58	0	
18	Chronic lymphadenitis	33	0	
19	Chronic lymphadenitis	10	0	
20	Chronic lymphadenitis	13	0	

LSa (L), lymphosarcoma (leukaemic); HD, Hodgkin's disease; RCSa, reticulum cell sarcoma; FL, follicular lymphoma; LP, lymphocytic predominance; MC, mixed cellularity; LD, lymphocytic depletion.

*Derived from pleural effusion of Hodgkin sarcoma. Chromosome analysis was done at the 50th transfer generation (350 d).

chromosomes were not found in the follicular lymphoma and the three chronic lymphadenitis.

Detailed analysis revealed that the marker was an abnormal chromosome 14 with a longer arm than usual, representing a tandem 14:14 long arm translocation (Fig. 1). Although some other chromosomal abnormalities were also found in the malignant lymphomas examined, none was common as the acrocentric marker involving chromosome 14.

The specific involvement of chromosome 14 in clonally proliferating human lymphocytes has been also demonstrated in ataxia-telangiectasia^{3,4}. The above findings therefore support the hypothesis by McCaw *et al.*⁴ that structural rearrangement of the long arm of chromosome 14 may be a step towards the development of lymphoid malignancies.

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Examination of ATCC stocks for HeLa marker chromosomes in human cell lines

INTRASPECIES contamination of cell cultures is a serious technical problem when more than one cell line is maintained in continuous serial subculture within a laboratory. Biochemical^{1–3} and immunological^{4,5} evidence has strongly suggested that many "cell lines" that are commonly used in the laboratory around the world have been contaminated with HeLa cells. Chromosome rearrangements resulting in specific "markers" provide a unique opportunity to monitor for intralinear specificity in HeLa and other human cells. Because certain cell lines in the American Type Culture Collection (ATCC) repository have already been shown to possess the type A (fast) G6PD characteristic of HeLa, we initiated a survey of these cell lines for the chromosome features described for HeLa cells^{6–9} using the trypsin-Giemsa banding technique¹⁰. Ten cell lines, including two HeLa lines, have been studied so far. Five of these lines have been found to contain marker chromosomes typical of HeLa cells. The non-HeLa cell lines have chromosome characteristics of their own which can also be used to monitor their purity.

The HeLa S3, ATCC-CCL 2.2 and HeLa, ATCC-CCL 2, have four distinct marker chromosomes (Figs 1a and 2). Marker 1 (M1) is formed by the fusion of centromere and the long arm of chromosome 1 and the long arm of chromosome 3. Marker 2 (M2) consists of the short arm of chromosome 3 and the long arm of chromosome 5. Marker 3 (M3) is an isochromosome of the short arm of chromosome 5, and usually occurs in more than one copy. Marker 4 (M4) consists of the long arm of chromosome 11 and an arm of chromosome 19. Our views on the origin of M4 differ from those of other investigators^{8–11,12}.

Marker chromosomes typical of HeLa cells (HM) are

found in the following cell lines: Detroit-6, ATCC-CCL 3; Chang liver, ATCC-CCL 13; KB, ATCC-CCL 17; AV₃, ATCC-CCL 21 and FL, ATCC-CCL 62. These lines are all epithelial in general morphology and possess the fast migrating variant (type A) of the isoenzyme glucose-6-phosphate dehydrogenase (G6PD). Detroit-6 cells lack HM1 but exhibit two copies of HM2, three or four copies of HM3, and HM4 is found occasionally. Even though this cell line was reported to have originated from a male Caucasian¹³, quinacrine fluorescence studies did not reveal the presence of the Y chromosome. In addition, there is a large isochromosome (LI) formed by the duplication of the long arm of chromosome 10 (Fig. 2). The Chang liver line exhibits all four HeLa-like markers, two copies of HM1, one copy of HM2, four copies of HM3 and a single copy of HM4 (Fig. 2). In the KB line, reported to have originated from a male Caucasian¹⁴, HM1 is missing but one copy of HM2, four copies of HM3 and a single copy of HM4 are present. There is a large submetacentric (LSC) marker chromosome probably formed by the translocation between an arm of chromosome no. 3 and the long arm of chromosome 7 (Figs 1c and 2). Quinacrine fluorescence studies also revealed the absence of the Y chromosome in this line. The AV₃ cell line does not reveal the presence of HM1 or HM2, however, three copies of HM3 and one copy of HM4 are noted. A large submetacentric chromosome (LSC), similar to the one found in KB is present (Fig. 2). All four marker chromosomes typical of HeLa cells are found in the FL line. Three copies of HM3 and two copies of HM4 are always present. In addition, these cells possess one large submetacentric chromosome, two large isochromes and one medium-sized isochromosome. The large submetacentric chromosome (LSC) is formed by chromosome 2 with a terminal duplication of the long arm. The large isochromosome (LI 1) is formed by the duplication of the long arm of chromosome 7 and the second large isochromosome (LI 2) is formed by the duplication of the long arm of chromosome 9. The medium-sized isochromosome is formed by the duplication of the long arm of chromosome 14 (Figs 1b and 2).

Marker chromosomes typical of HeLa cells are not found in the following cell lines: RPMI 2650, ATCC-CCL 30 (Fig. 1d); RD, ATCC-CCL 136 and Detroit 562, ATCC-CCL 138. These lines also have an epithelial morphology but possess the slow migrating variant (type B) of G6PD. The RPMI 2650 cells possess the Y chromosome as determined by the quinacrine fluorescence technique. Examination of RD and Detroit 562 lines using the same technique did not reveal the Y chromosome which would be expected since they are of female origin. Detroit 562 cells contain a large submetacentric marker chromosome probably formed by chromosome 5 with a terminal duplication of the long arm.

Table 1 summarises our findings. The absence of the Y chromosome, possession of G6PD type A and the presence of HeLa marker chromosomes provide strong evidence that Detroit-6 and KB are cross contaminated with HeLa cells. Although the donor sex and racial origin of the Chang liver and FL are unknown, the fact that they both possess all four HeLa marker chromosomes indicates the possibility that these lines also are cross contaminated with HeLa cells. The AV₃ which is of negro origin and donor sex unknown, likewise possesses HeLa marker chromosomes. The absence of certain HeLa marker chromosomes in some of these cell lines may be due to partial contamination with HeLa cells probably through somatic cell hybridisation. The other possibility is that the gain or loss of these markers, after their initial appearance in the HeLa-contaminated parental stock, could represent no more than random effects of different culture conditions in different laboratories. The occurrence of identical large submetacentric chromosomes (LSC) in both the KB and

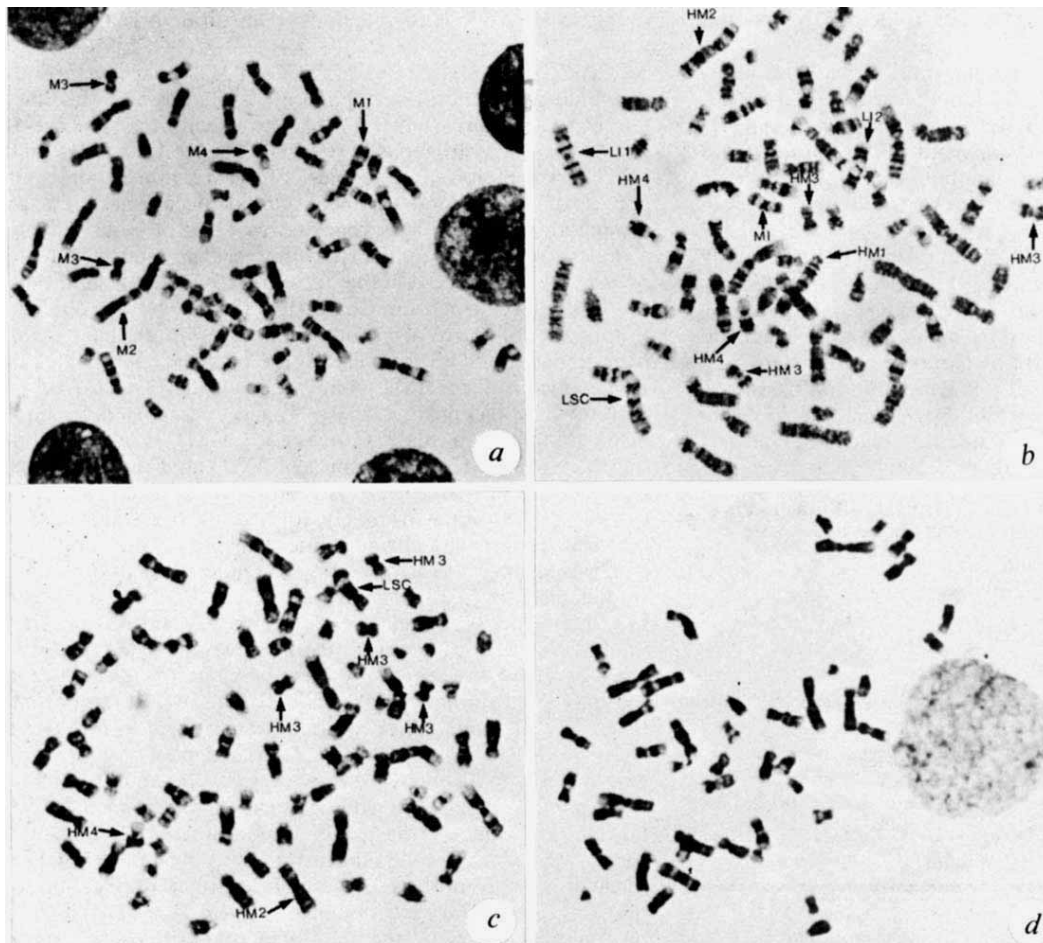


Fig. 1 *a*, Trypsin-Giemsa banded metaphase chromosomes of HeLa, S3, ATCC-CCL 2.2. Arrows indicate marker (M) chromosomes. *b*, Trypsin-Giemsa banded metaphase chromosomes of FL, ATCC-CCL 62. Arrows indicate HeLa-like markers (HM), large and medium-sized isochromosomes (LI 1, LI 2 and MI) and a large submetacentric chromosome (LSC). *c*, Trypsin-Giemsa banded metaphase chromosomes of KB, ATCC-CCL 17. Arrows indicate HeLa-like markers (HM) and a large submetacentric chromosome (LSC). *d*, Trypsin-Giemsa banded metaphase chromosomes of RPMI 2650, ATCC-CCL 30. Note the lack of HeLa-like marker (HM) chromosomes.

AV₃ indicates that these two cell lines might have been cross contaminated with each other or that these two identical markers arose independently of each other. At the time when these certified cell lines were deposited in the ATCC Animal Cell Repository as frozen ampoules, technology was not available to check for intraspecific cell contamination. With modern techniques it is now possible to monitor cell lines for cross contamination of this kind. Since we have examined these lines carefully within two passages beyond that of the frozen seed stock, using trypsin-Giemsa banded marker chromosomes, these lines were undoubtedly already contaminated with HeLa cells before reaching the ATCC Repository. On the other hand, lack of HeLa-like markers coupled with the findings for G6PD and the Y chromosome clearly indicate that RPMI 2650, RD and Detroit 562 are not cross contaminated with HeLa cells (Table 1).

Thus it seems that investigators who culture more than one cell line in the laboratory at the same time run a high risk of cross contaminating their cultures. Consequently, all cell lines should be periodically and frequently monitored through isoenzyme analysis, chromosome banding and immunological techniques to prevent intra- or interspecies contamination.

Note added in proof: The above information is already incorporated in a new edition of the ATCC catalogue (ATCC Catalogue of Strains II, first edition, 1975). A discussion of the intraspecific cell contamination problem appears in the introduction and all of the ATCC stocks which are known to bear HeLa-like characteristics are clearly marked in the catalogue. We are currently examining all other ATCC stock of human cell lines for chromosome features characteristic of HeLa cells and the information will be made available in subsequent publications.

Since the submission of this manuscript for publication

Fig. 2 Trypsin-Giemsa banded marker chromosomes of cell lines examined.

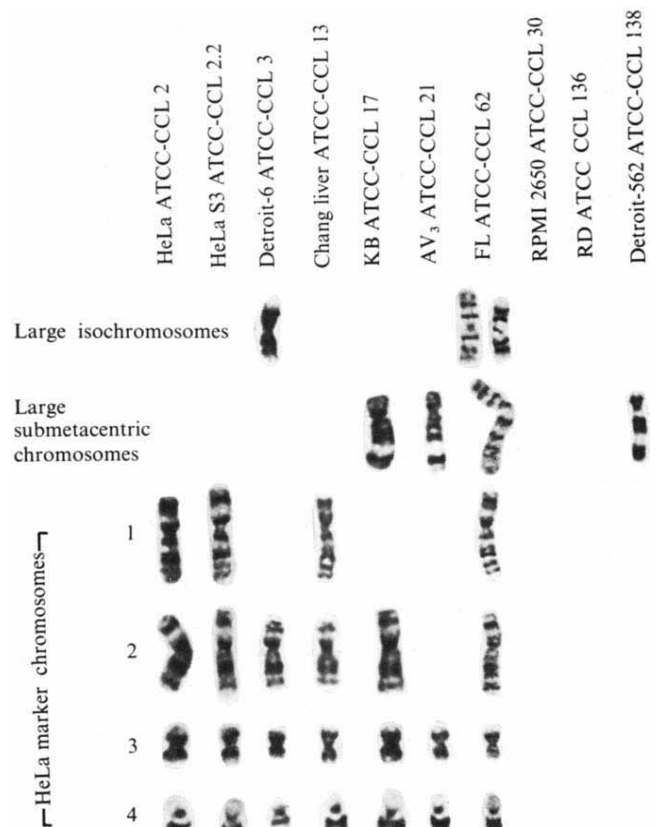


Table 1 ATCC stocks of human cell lines examined for HeLa marker chromosomes

Cell Line	Deposited in ATCC Repository		Tissue of origin	Racial origin	Type of G6PD	Sex of donor	Y chromo- some	Passage no.	Chromosome		Marker chromosomes HeLa markers					Other markers
	Date	Passage no.							no.	Range	No. 1	No. 2	No. 3	No. 4		
HeLa (ATCC-CCL 2)	6-19-63	?	Cervical carcinoma	Negro	A	F	—	?	84	58-179	+	+	4-5*	2*	—	
HeLa S3 (ATCC-CCL 2.2)	5-15-73	?	Clone of CCL 2	Negro	A	F	—	?	68	51-74	+	+	2*	+	—	
Detroit 6 (ATCC-CCL 3)	6-19-63	160	Sternal bone marrow (Patient with carcinoma of lung)	Caucasian	A	M	—	160	67	51-75	—	2*	3-4*	±	LI†	
Chang Liver (ATCC-CCL 13)	3-29-63	255	Normal liver	?	A	?	—	257	70	61-82	2*	+	4*	+	—	
KB (ATCC-CCL 17)	6-19-63	361	Epidermoid carcinoma of oral cavity	Caucasian	A	M	—	362	75	53-80	—	+	4*	+	LSC†	
AV ₃ (ATCC-CCL 21)	9-17-63	270	Amnion	Negro	A	?	—	272	77	58-95	—	—	3*	+	LSC†	
FL (ATCC-CCL 62)	1-17-66	?	Amnion	?	A	?	—	?	71	68-142	+	+	3*	2*	LSC† 2 LI† MI§	
RPMI 2650 (ATCC-CCL 30)	11-25-66	22	Pleural effusion (Patient with carcinoma of nasal septum)	Caucasian	B	M	+	28	46	43-92	—	—	—	—	—	
RD (ATCC-CCL 136)	5-13-70	32	Rhabdo- myosarcoma	Caucasian	B	F	—	45	51	49-97	—	—	—	—	—	
Detroit 562 (ATCC-CCL 138)	11-24-69	43	Pleural effusion (Patient with carcinoma of pharynx)	Caucasian	B	F	—	44	64	58-128	—	—	—	—	LSC†	

*No. of copies when more than one.

†Large submetacentric chromosomes (LSC).

‡Large isochromosomes (LI).

§Medium sized isochromosome (MI).

five more human cell lines have been examined and found to possess HeLa marker chromosomes. These cell lines are Minnesota EE, ATCC-CCL 4; L-132, ATCC-CCL 5; Detroit-98, ATCC-CCL 18; NCTC 2544, ATCC-CCL 19; and WISH, ATCC-CCL 25.

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Vertical transmission of tumour resistance in guinea pigs

BOTH intact bacillus Calmette-Guerin (BCG)¹⁻³ and the methanol extraction residue (MER) fraction⁴ of BCG have had extensive application in tumour immunotherapy. Treatment with MER nonspecifically stimulates both the cellular and humoral immune systems^{4,5}, and has been successful in tumour immunotherapy in a number of animal models^{6,7}. Experiments in our laboratories, using animals obtained from the Weizmann Institute, Rehovot, Israel, have shown that about 40% of strain 2 guinea pigs injected with MER or with the supernatant of sonically-disrupted BCG (BCG-SS), 1-2 months before intradermal inoculation with 10⁶ cells of the transplantable line 10 hepatocarcinoma^{8,9}, are resistant to the growth of this tumour¹⁰. Similarly, injection of MER into developing tumour nodules 7d after tumour cell implantation results in complete tumour regression in approximately 40% of the animals tested (M.A.W., P.M. and D.W.W., unpublished).

Previous studies have demonstrated that specific antibodies can traverse the placental barrier from maternal to foetal guinea pigs¹¹. About 50% of the offspring of BCG-SS-treated female guinea pigs that became resistant to line 10 tumour were themselves resistant to challenges with line 10 tumour cells¹².

We became interested in whether a similar transfer of protection against this tumour might also occur following treatment with MER, and what the mechanism of this vertical transmission might be. Line 10 hepatocarcinoma (B. Zbar, National Cancer Institute, Bethesda, Maryland) was maintained in ascites form by intraperitoneal passage in strain 2 guinea pigs (bred at Weizmann Institute). Tumour cells were collected and prepared for intradermal inoculation as described previously¹⁰. Nine tumour-free female guinea pigs, which had been treated with single doses of MER (0.5–1.0 mg) (Department of Drug Research and Development, National Cancer Institute) or BCG-SS (50 µg N) before or after tumour cell implantation and which were resistant to further challenge (10⁷ cells), were mated with normal males at least 60 d after their initial tumour cell challenge (10⁶ cells). The newborn guinea pigs of these matings were inoculated intradermally with 10⁶ tumour cells when approximately 30 d old and numbers of survivors and mean days of death recorded (Table 1). Age-matched offspring of seven normal guinea pigs served as controls. Complete tumour regression was observed in 11 of the 26 experimental animals tested (42%), whereas a considerable delay in onset of death was observed in nine others (experiments 2, 5, 6 and 7). No differences were observed between the capacities of male and female newborns to survive tumour challenge. Growing tumour

nodules were observed in all guinea pigs by 4 d after tumour cell implantation. In those animals which developed complete resistance to the tumour, these nodules became progressively smaller from about 7 d after tumour challenge and eventually disappeared. In the case of many guinea pigs which eventually succumbed, tumour growth was markedly retarded at the site of implantation, and it was only following the appearance of palpable metastatic lesions in the regional lymph nodes that progressive tumour enlargement and death ensued. In contrast, rapid tumour growth at the site of initial challenge followed by death was observed with each of 19 age-matched controls ($P < 0.001$; χ^2 test). In three cases (Table 1, experiments 8–10), the tumour resistant female offspring of MER-treated animals were themselves mated with normal males and the third generation guinea pigs challenged with 10⁶ tumour cells as described above. Vertical transmission of tumour resistance was again observed; three of the eight third generation offspring survived tumour challenge as opposed to none of the six age-matched controls.

To evaluate whether a transplacental transfer of immune factors was taking place, some of the maternal tumour-resistant animals of Table 1 and their offspring were skin tested, 3 d before and 3 weeks after tumour cell challenge, with 0.1 ml each of purified protein derivative of *Mycobacterium* (PPD, Ministry of

Table 1 Vertical transmission of tumour resistance

Experiment	History of mother	Age of newborns at challenge (d)	Number in litter	Number of survivors	Mean day of death of non-survivors
1	BCG-SS immunoprophylaxis cure	30	2	2	—
	Controls	30	2	0	54
2					
Litter a	MER immunoprophylaxis cure*	30	2	0	71
Litter b	MER immunoprophylaxis cure	32	1	1	—
	Controls	28	2	0	54
3	MER immunoprophylaxis cure*	29	3	0	47
	Controls	30	2	0	52
4					
Litter a	MER immunotherapy cure	31	4	1	40
Litter b	MER immunotherapy cure	28	1	1	—
	Controls	29	2	0	55
5	MER immunotherapy cure	25	4	1	83
	Controls	27	4	0	55
6					
Litter a	MER immunotherapy cure	29	3	2	81
Litter b	MER immunotherapy cure	31	2	1	79
	Controls	30	4	0	61
7	MER immunotherapy cure	28	4	2	78
	Controls	28	3	0	58
8	Female survivor of experiment 1	28	3	0	69
	Controls	32	2	0	62
9	Female survivor of experiment 2, litter b	31	2	1	64
	Controls	29	2	0	58
10	Female survivor of experiment 4, litter b	30	3	2	64
	Controls	30	2	0	60
	Total number of experimental animals		34	14	65
	Total number controls†		25	0	—

*Same mother; litters born 4 months apart.

†Significance of comparison: experimentals-controls, $P < 0.001$.

Table 2 Effect of cross rearing of newborn guinea pigs on tumour survival

Offspring of	Nursed by	Number in litter	Number of survivors	Mean day of death of non-survivors
Tumour-resistant mother 1	Normal mother 1	2	2	—
Tumour-resistant mother 2	Normal mother 2	4	2	61
Tumour-resistant mother 3	Normal mother 3	3	0	65
Normal mother 1	Tumour-resistant mother 1	2	0	63
Normal mother 2	Tumour-resistant mother 2	2	0	59
Normal mother 3	Tumour-resistant mother 3	4	0	66
Tumour-resistant male 1	Normal mother 4	4	0	63
Tumour-resistant male 2	Normal mother 5	3	0	68
Tumour-resistant male 3	Normal mother 6	3	0	59
Tumour-resistant male 4	Normal mother 7	4	0	62

Agriculture, Weybridge, UK), soluble tumour antigen extract (SA-10, prepared by 3 M KCl extraction as described previously¹³), soluble normal liver antigen extract (SA-N, prepared as above¹³), and isotonic saline (NaCl). Areas of induration were measured after 24 and 48 h. Tumour-resistant mothers showed significant delayed cutaneous hypersensitivity (DCH) to both PPD and SA-10 but not to either SA-N or NaCl. There was no significant response by either tumour-resistant or susceptible offspring to these antigens before challenge with tumour cells. Three weeks after challenge, however, all the offspring of tumour-resistant and control mothers had DCH reactions to SA-10. Thus, DCH to SA-10 and PPD did not seem to be transmitted transplacentally to these newborn guinea pigs, and the development of DCH to SA-10 after tumour challenge was not related to their ultimate survival.

The possibility that vertical passage of immune serum elements might occur was assessed. Each of three MER-treated and tumour-resistant guinea pigs were injected intramuscularly with 10⁷ tumour cells to stimulate both cellular and humoral anti-tumour immunity. These animals, all of which showed strong DCH reactivity to SA-10 (10 µg), were bled 3 and 7 days after immunisation, and 1 ml aliquots each of serum, plasma or whole blood cells were passively transferred by cardiac puncture to ten normal 25-d-old recipients. The latter were challenged 7 d later with 10⁶ tumour cells. No retardation of either tumour growth or onset of death was observed in any of these animals.

To study the possible role of the milk of nursing mothers as a source of tumour resistance, the newborn offspring of each of three MER-immunotherapy-cured females and three female controls were interchanged as indicated in Table 2. The 17 newborn guinea pigs in this 'criss-cross' experiment were challenged with 10⁶ cells when about 30 d old. Four of the nine offspring of MER-treated, tumour-resistant mothers were themselves resistant to growth of the line 10 hepatocarcinoma, in spite of having been suckled on normal lactating females. By contrast, tumour-resistant females seemed to be unable to transfer resistance by nursing to any of the eight offspring of control mothers. This finding is in agreement with the observation that guinea pig IgG antibodies are not transmitted through the milk¹⁴. Parallel experiments (Table 2) showed that none of the 14 newborn guinea pigs born to normal mothers but fathered by MER-treated tumour-resistant males resisted challenge with line 10 cells.

In summary, vertical transmission of resistance to the transplantable guinea pig line 10 hepatocarcinoma from MER-treated tumour-resistant mothers has been demonstrated. The nature of the mechanisms responsible for this protection remains to be elucidated. It is clear, however, that they are not related to factors transmitted through the milk. The fact that a significant proportion of the third generation offspring of resistant guinea pigs are also tumour-resistant makes it unlikely that this phenomenon is attributable to any lingering effect of MER or BCG-SS. Tests for DCH did not reveal evidence of cellular anti-tumour immune activity in the offspring of tumour-immune mothers, before skin challenge with line 10 hepatocarcinoma cells. Nevertheless, the considerations that small quantities of anti-tumour antibodies and/or specifically sensitised small lymphocytes may be transported across the placental barrier cannot be ruled out and are presently under investigation.

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Gene that controls initiation of chromosome replication and prophage induction in *Bacillus subtilis*

THE mechanism of initiation of the chromosome replication cycle in bacteria is beginning to be elucidated by genetic and biochemical studies¹⁻³. But little is known about what controls the initiation frequency which has a key role in regulating the rate of DNA synthesis in response to the change in bacterial growth rate^{4,5}. The existence of such a regulatory entity—initiator—synthesised in harmony with the increase in cell mass or the change in the cell or replication cycle, has been hypothesised^{6,7}. We have investigated the nature of the hypothetical regulator, based on the assumption that the induction of prophage and the initiation of host cell chromosome replication would be controlled by a common regulator directly or indirectly. The assumption is based on observations that the inducibility of prophages and the initiation frequency of chromosome replication share common features in their responses to the bacterial growth rate and to the inhibition of DNA replication^{4,5,8-10}. We assumed that the regulatory material, substance X, accumulates in the cell depending on growth rates and is consumed to initiate each replication cycle, and

that an unbalanced accumulation of X caused by the inhibition of DNA synthesis would provoke prophage induction. If this assumption is correct it should be possible to isolate conditional mutants in which both the initiation of chromosome replication and prophage induction are prevented in non-permissive conditions.

Induction of a temperate phage SPO2 in *Bacillus subtilis* by 6-*p*-hydroxyphenylazouracil (HPUra)¹¹ was used as a test system because the uracil analogue inhibited semi-conservative DNA replication by interacting specifically with DNA polymerase III of *B. subtilis*, without affecting either the repair synthesis¹² of the cell or the replication of the phage DNA. Most of the commonly used inducing agents, such as ultraviolet irradiation, mitomycin C (mit C) and nalidixic acid are not suitable for this test because they cause damage in the DNA molecule, which in turn may affect prophage inducibility^{13,14}.

B. subtilis 168 (*trp*⁻), CRK1000, was lysogenised with the phage SPO2 and mutated with nitrosoguanidine¹⁰. The resultant mutagenised lysogens were grown in a rich Lp medium¹⁵ at 30 °C and then heated to 45 °C with the addition of HPUra 5 min after heating. After 60 min of incubation at 45 °C survivals were collected on a filter paper and washed to remove HPUra. From these cells temperature-sensitive (*ts*) mutants were isolated by a conventional replica method. Properties of 133 *ts* mutants isolated in several experiments are summarised in Table 1. Thirty-seven mutant lysogens which showed both temperature-sensitive growth and temperature-sensitive induction (no induction by HPUra at 45 °C were isolated either spontaneously or after treatment with nitrosoguanidine. A double selection by repeated HPUra induction at 47 °C

isolated this type of mutant. The mutation in the host chromosome was demonstrated directly by introducing the *ts* character into a recipient, *thy*⁻ cell. The resultant transformants carried *ts* and *thy*⁻ characters and therefore DNA synthesis in them was measured by ³H-thymidine incorporation. Ten out of 31 transformed mutant strains

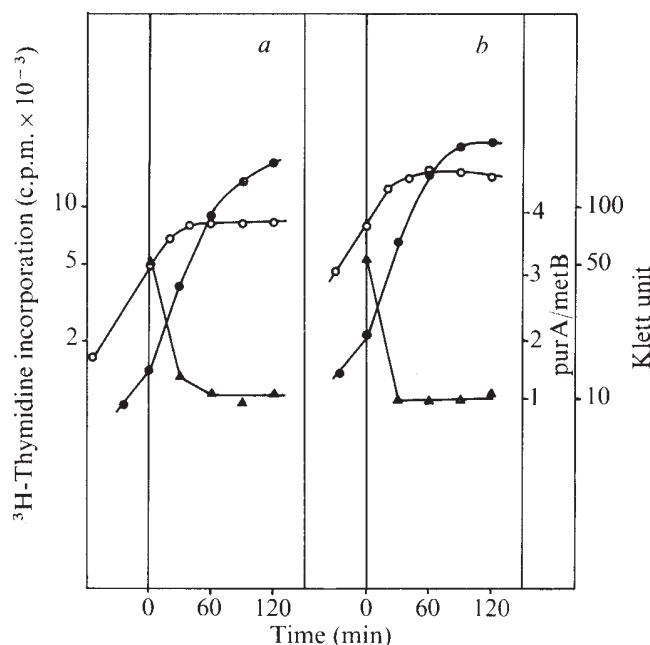


Fig. 1 Growth and DNA synthesis of *dna-ts* initiation mutant, CRK2605 (*ts*1315) and CRK2001 (*ts*27). Cells of CRK2605 or CRK2001 were grown in Lp medium containing necessary amino acid and ³H-thymidine (0.5 μ Ci per 5 μ g per ml) until an early exponential phase when the temperature of the culture was shifted quickly to 47 °C. At various times before and after the shift, 0.5 ml samples were taken to measure radioactivities incorporated into the DNA fraction after incubation in 0.2 M NaOH at 80 °C for 30 min, followed by precipitation in 10% trichloroacetic acid. In parallel with this measurement DNA was isolated from 5-ml samples and its transforming activities for adenine and methionine were determined. Marker frequency analysis was done as before¹⁸. A mutant strain CRK3000 (*leu*⁻, *metB*⁻, *purA*⁻, *hisA*⁻) was used as recipient. *a*, CRK2605; *b*, CRK2001. $\circ$, ³H-thymidine incorporation; $\bullet$, cell turbidity in Klett unit; $\blacktriangle$, *purA/metB*.

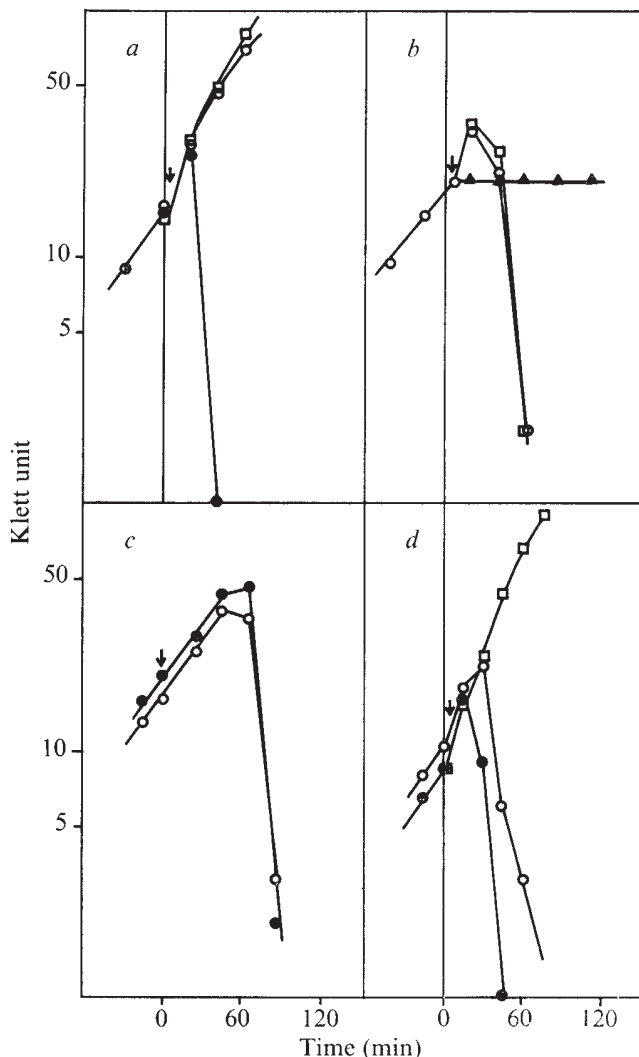


Fig. 2 Effects of HPUra and mitC on induction and infection of SPO2 in a mutant carrying *ts-dna-ind* mutation, CRK2605. CRK2605 or its lysogen, CRK2605(SPO2), was grown in complete Lps medium (which is Lp plus MgSO_4 , 5 mM, and MnCl_2 , 0.5 mM) at 30 °C. At an early exponential phase, cells were treated as below and the turbidity was measured using a Klett colorimeter. After the last turbidity measurement, all the culture was added to NaN_3 (final 25 mM) and stored at 4 °C overnight to determine the phage titre by the method of Okubo and Romig¹⁹. *a*, CRK2605(SPO2) was heated to 47 °C at time zero and drugs were added 5 min later. Incubation was continued at 47 °C. $\square$, No drug, 4.2×10^6 plaque-forming units per ml (PFU ml^{-1}) in the lysate; $\circ$, with HPUra (70 μM), 2.5×10^7 PFU ml^{-1} ; $\bullet$, with mit C (1 $\mu\text{g ml}^{-1}$), 6.3×10^8 PFU ml^{-1} . *b*, CRK2605 was heated to 47 °C at time zero and the phage SPO2 (multiplicity of infection 0.6) was added to the culture 5 min after heating, with or without drug. Incubation was continued at 47 °C. $\square$, No drug added, 2.4×10^8 in the lysate; $\circ$, with HPUra, 2.1×10^8 PFU ml^{-1} ; $\blacktriangle$, with HPUra and rifampicin (2 $\mu\text{g ml}^{-1}$), 7×10^8 PFU ml^{-1} . *c*, CRK2605(SPO2) was added with drugs at time zero and incubation continued at 30 °C. $\square$, With HPUra, 1.9×10^8 PFU ml^{-1} in the lysate; $\circ$, with HPUra, 2.1×10^8 PFU ml^{-1} ; $\bullet$, with mit C, 1.7×10^8 PFU ml^{-1} . *d*, For comparison, the mutant lysogen carrying *ts*27, CRK2001(SPO2), was grown at 30 °C, heated to 47 °C and added with drugs as in (*a*). $\square$, No drug addition, 1.0×10^8 PFU ml^{-1} in the lysate; $\circ$, with HPUra, 4.7×10^8 PFU ml^{-1} ; $\bullet$, with mit C, 3.9×10^8 PFU ml^{-1} .

Table 1 Isolation of *ts* mutants of *B. subtilis* from surviving SPO2 lysogens after inducing treatments at high temperature

Mutagenised by NTG	Selection methods	Lysogenic cells (<i>trp</i> ⁻ (SPO2))		Transformants (<i>thy</i> ⁻ , <i>ts</i>)		Total
		<i>ts</i> mutants	HPUra-induction <i>ts</i> mutants	<i>dna-ts</i> mutants	Others*	
				Initiation	Elongation	
+	—	23	3	0	0	3
+	HPUra	74	24	9	2	21
+	HPUra-ultraviolet	25	4	0	0	1
—	HPUra-HPUra	11	6	1	0	6
Total		133	37	10	2	31

The SPO2 lysogen of *B. subtilis* 168 (*trp*⁻), CRK1000 (SPO2), was grown in Lp medium¹⁵, and treated with nitrosoguanidine (NTG) at 30 °C as before¹⁰. NTG-treated cells were washed and grown at 30 °C in minimal medium Cg (ref. 18) to minimise prophage induction after NTG treatment. After about 60 min cells began to increase when they were mixed with 20% glycerol and frozen in liquid nitrogen. Mutagenised cells were thawed immediately and grown in Lp medium at 30 °C for one generation. One sample (column 1) was plated directly on BHI agar¹⁰ without further treatment. Other cells were heated to 45 °C, added to HPUra (70 µM) after 5 min and incubated for 60 min at 45 °C. Cells lysed at about 45 min. Surviving cells were collected by filtration, washed and then plated on BHI agar (column 2). In two samples (columns 3 and 4), survivals from the first HPUra treatment were grown in Lp medium at 30 °C and then heated to 45 °C. After 5 min at 45 °C cells were either added with HPUra (70 µM) or irradiated with ultraviolet light at 4 µW cm⁻² for 2 min. Cells were further incubated at 45 °C for 60 min and cells remaining after lysis were filtered, washed and plated on BHI agar. For the sample in the last column cells grown in Lp medium at 30 °C were used without NTG treatment. Cells plated on BHI agar plate after these various selections were grown at 30 °C and the colonies appearing after 12 h were replica plated on BHI and incubated at 45 °C. Colonies that failed to grow at 45 °C were isolated as *ts* mutants. *ts* lysogens thus obtained were grown in Lp medium at 30 °C and HPUra (70 µM) was added at an early exponential phase either at 30 °C or 5 min after heating to 45 °C. Cell lysis and phage production (detected by a spot test) were followed at both temperatures. The number of *ts* mutants that lysed and produced phage at 30 °C and did not lyse and produced few phages at 45 °C are listed. DNA was isolated from the HPUra-induced *ts* mutants (total 37) as described before¹⁸. Competent cells of CRK1500, *thy*⁻, *leu*⁻, were transformed with these DNA samples of saturated concentration¹⁸. From *leu*⁺ transformants, *ts* transformants were selected and immunity to and infectivity by SPO2 were examined to confirm that the SPO2 genome was not co-transformed with *ts* markers. Of the 37 mutants, 31 were transformed successfully. The transformants (*ts*, *thy*⁻) were grown at 30 °C in the Lp medium containing ³H-thymidine (0.5 µCi per 5 µg per ml) and DNA synthesis and growth were determined by ³H-thymidine incorporation and turbidity measurement respectively, after heating the exponentially growing cell to 47 °C. In initiation mutants, the residual amount of DNA synthesis was 50–100%, equivalent to the DNA present at the time of heating. The same amount was less than 20–30% in the elongation mutants.

* These include the mutants in which more than 100% of DNA was synthesised at 47 °C and those in which both DNA synthesis and growth were inhibited at 47 °C.

were found to be *dna-ts* initiation mutants. Marker frequency analysis during the incubation of these mutants at 47 °C clearly showed that the initiation of new rounds of replication was prevented immediately after heating (Fig. 1).

As expected, when these mutants were lysogenised with a wild-type SPO2 the prophage was induced by HPUra at 30 °C but not at 47 °C, as shown by cell lysis (Fig. 2), the phage titre liberated into the medium (legend to Fig. 2) and the incorporation of ³H-thymidine in the presence of HPUra, which is a direct indication of the replication of the SPO2 DNA (Fig. 3). Data in Figs 2 and 3 show that (1) the sensitivity of chromosome replication to HPUra was not altered by the mutation, (2) the expression of early genes and the autonomous replication and maturation of SPO2 occurred normally at 47 °C in the presence of HPUra, as indicated by a successful infection by SPO2 and induction by mit C at 47 °C. Therefore we conclude that the change in the prophage from a repressed to a derepressed state caused by the cessation of chromosome replication by HPUra is prevented specifically at the non-permissive temperature.

From this evidence we conclude that the mutation in this group of *ts* mutants affects, at the non-permissive temperature, both the initiation of chromosome replication and the prophage induction by HPUra. We call them collectively *ts-dna-ind* mutants. No *ts-dna-ind* mutant was isolated when ultraviolet treatment was used at the second selection cycle in place of HPUra, and mit C

caused SPO2 induction in the mutants at 47 °C even in the presence of HPUra, indicating that ultraviolet light and mit C caused induction through a mechanism at least partly different from that of HPUra. These mutants carried a single mutation and the induction by HPUra occurred at 47 °C in *ts*⁺ revertants. Furthermore, both initiation of chromosome replication and the induction of SPO2 by

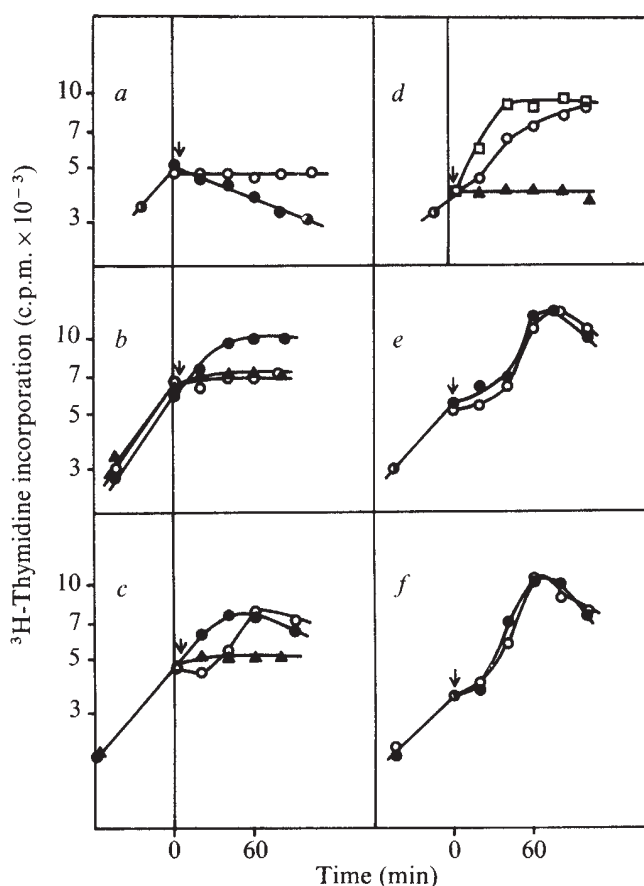


Fig. 3 Induction and infection of the phage SPO2 in *dna-ts* initiation mutants assayed by ³H-thymidine incorporation in the presence of HPUra. Two *dna-ts* initiation mutants, CRK2605 and CRK2001, and their SPO2 lysogens were grown in complete Lps medium containing ³H-thymidine (0.5 µCi per 5 µg per ml) at 30 °C. At an early exponential phase, cells were treated as shown below and DNA synthesis was measured as described in the legend to Fig. 1. □, Without drug; ○, with HPUra; ●, with mit C and HPUra; ▲, with HPUra and rifampicin. *a*, CRK2605; *b*, CRK2605(SPO2); *c*, CRK2001(SPO2); *d*, CRK2605. The cell was cultured at 30 °C and then the temperature of the culture was shifted quickly to 47 °C. After 5 min cells were added to the drugs and in (*d*) infected with the phage SPO2 (multiplicity of infection 0.6) simultaneously; *e*, CRK2605(SPO2) was cultured at 30 °C; *f*, CRK2001(SPO2) was cultured at 30 °C.

HPUra occurred when mutant cells (non-lysogen and SPO2 lysogen, respectively) were treated at 47 °C for 60 min and then cooled at 30 °C. Chromosome replication was re-initiated even in the presence of chloramphenicol, suggesting that the *ts* component was readily reactivated at 30 °C (data not shown).

Genetically it is interesting to ask whether these *dna-ind* mutations are clustered in one gene or dispersed in many genes. Recombination tests using two mutants carrying the *dna-ind* mutations as recipients revealed that the 10 *ts-dna-ind* mutations were not identical but closely linked, having recombination indices of 0–0.3, suggesting that they are located within one gene¹⁶. Furthermore, these mutations were tightly linked to *dna-ts27*, a mutation carried by a *ts-dna* initiation mutant isolated in our laboratory¹⁷. Phenotypes of the mutant with respect to the *ts* character of the initiation of the chromosome replication were indistinguishable from *dna-ind* mutations (Fig. 1). SPO2, however, was induced by HPUra in the *ts27* mutant even at 47 °C (Figs 2 and 3). These results suggest that the initiation of chromosome replication and prophage induction are affected independently by the *dna-ind* gene product. A single mutation in this gene may alter either one of the two functions or both functions simultaneously.

We assume that X has dual functions in the initiation of chromosome replication and prophage induction. This is suggested by the general resemblance between the two events in terms of their responses to bacterial growth rates. The highly selective concentration of the specific type of the *dna-ts* initiation mutant by the selection method used in this experiment revealed close relationship between the two events. Furthermore, the finding of the *dna-ind* gene and pleiotropic effects of its mutations support the existence of the suggested substance X. The relationship between the product of this gene and X is unclear at present but the simplest interpretation is that the product is X itself.

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Differentiation of B and T mouse lymphocytes in cell suspension and smears

THE differentiation of B and T lymphocytes by scanning electron microscopy and, in live suspensions, by light microscopy has led to controversy about their different surface characteristics^{1,2}. They can be demonstrated serologically by the presence or absence of specific antigen markers in various animal species, including man. B cells can be re-

cognised among mouse lymphocytes by surface immunoglobulin staining under fluorescent microscopy³. The surface morphology of lymphocytes has been distinguished by light microscopy using Nomarski differential interference⁴. We have now used the Hoffman modulation contrast microscopy system^{6,7} and confirmed that B and T lymphocytes have different surface characteristics. This technique makes it possible to observe both live cells and air-dried smears, and we have demonstrated the feasibility of restoring the distinct surface characteristics in blood smears.

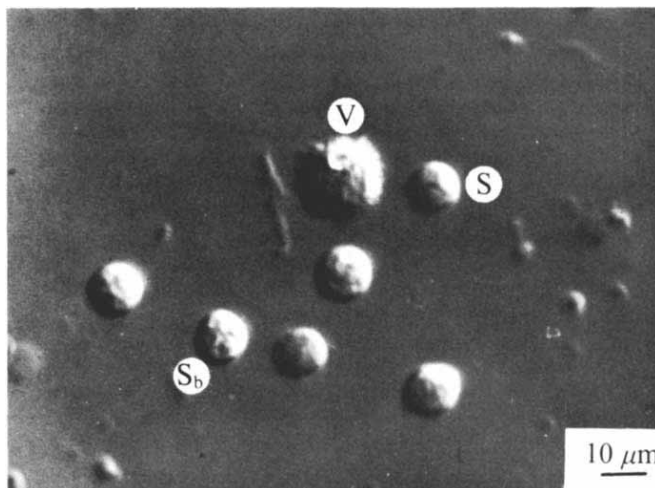
C57BL/6, BALB/c and Dublin (ICR) Swiss mice, 4–8 weeks old, and of both sexes, were used. Peripheral blood was collected from the orbital sinus in 24 heparinised haematocrit tubes, and pooled in a syringe containing heparin (50 U). Pooled blood was layered on a Ficoll-Hypaque mixture for density gradient separation of lymphocytes and platelets by Boyum's method⁸. This layer was then removed and centrifuged at 400g and resuspended in Eagle's minimum essential medium (MEM) with or without 5% foetal calf serum (FCS).

Spleen and thymus from individual mice were pressed separately and gently through a stainless steel wire mesh in 5 ml of MEM with or without 5% FCS, followed by passage through a 26-gauge needle.

Fluorescein-conjugated anti-mouse IgM was obtained from Meloy Laboratories. Lymphocyte pellets obtained from a single spleen and thymus, or density-gradient-separated peripheral blood lymphocytes, were sedimented at 400g for 5 min and resuspended in 0.2 ml of MEM, then combined with 0.2 ml of the fluorescein-conjugated anti-serum at a protein concentration of 0.8 mg ml⁻¹. The reaction mixture was incubated at 37 °C for 30 min with occasional shaking. Cells were washed three times in MEM only and resuspended in 1 ml of MEM containing FCS and applied to a microscope slide chamber made of two No. 1 glass coverslips mounted on the slide with Corning silicone vacuum grease. These supported a third coverslip, No. 1 1/2 thickness, making a thin chamber closed on two sides⁹. The chamber was filled by applying the cell suspension to one of the open sides, and inverted for 1–5 min to allow the cells to settle on the coverslip. When the chamber was reversed, many cells were hanging from the underside of the coverslip. Before examination, all cell suspensions were tested for cell viability by Trypan blue exclusion and found to contain more than 90% viable cells.

Peripheral blood obtained for the cell suspensions was also used for blood smears. Duplicate blood smears were

Fig. 1 Thymus suspension from Dublin Swiss mouse showing the three types of surface characteristics of lymphocytes: villous (V), smooth (S), smooth, with a broad base surface difference (S_b) (×40 achromat objective and ×10 ocular, Kodak 35-mm film No. 5032).



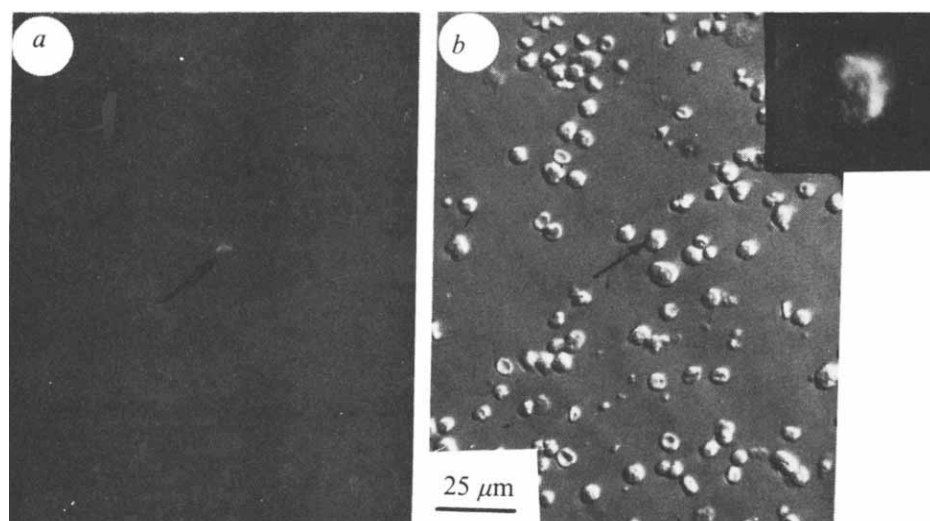


Fig. 2 *a*, Spleen suspension from Dublin Swiss mouse tagged with fluorescein isothiocyanate anti-mouse IgM; crescent-shaped fluorescence from a villous lymphocyte, *b*, modulation contrast of same field. The arrow points to the same cell ($\times 40$ achromat objective $\times 3.3$ ocular, Polaroid film Type 107 and Kodak SO 410 35-mm film (inset)).

prepared from individual mice, one acted as a control slide stained with Wright's stain for differential white blood cell determination, the other for Hoffman modulation contrast visualisation. The unstained smear was processed within 24 h by immersion in physiological buffered saline at 20 °C for 30 s for red cells to lysis. A coverslip was then applied to the wet surface, excess fluid removed and the edges sealed with nail polish. Differential counts were made for lymphocytes, granulocytes and monocytes.

The Hoffman modulation contrast system was adapted to an ordinary Nikon bright-field transmission microscope, and to the Olympus reflected light fluorescent system on the Vanox transmission microscope by Modulation Optics Inc. The system involves a modulator placed off-centre in the back focal plane of the objective and an off-centre slit beneath the condenser. This creates intensity contrast of phase gradients, producing a three-dimensional image. The objective with the modulator is used with both reflected fluorescent and transmitted modulation contrast without degradation of the fluorescent image.

Suspensions of live lymphocytes from the peripheral blood, spleen and thymus of mice, examined with a $\times 40$ achromat, objective, revealed two types of lymphocyte, some with fine, villous projections and others which were smaller, but with smooth surfaces (Fig. 1). There were also cells with essentially smooth surfaces, but which contained broad based projections (Fig. 1). When these suspensions were examined in the enclosed slide chamber for up to 1 h there was no essential change in their surface characteristics. Most lymphocytes in the lymphoid organ suspensions examined had smooth surfaces. Lymphocytes with villous

surfaces were fewer and varied in quantity with 0.2–2% in the thymus, an average of 13% in the peripheral blood and 23.2% in the spleen (Table 1).

Surface morphology of lymphocyte suspensions from the thymus incubated with fluorescein-conjugated anti-mouse IgM revealed less than 1% positive cells. When transmitted modulation contrast microscopy was directed to the same field of view, all fluorescent lymphocytes had villous surfaces. Less than 1% were villous and negative and all smooth lymphocytes were negative (Table 1).

Fluorescent cells revealed typical staining, with Ig limited to the surface in the form of a crescent or complete peripheral rim, or a stippled area. Specimens preincubated with unlabelled anti-mouse gamma globulin did not fluoresce when incubated with fluorescein-conjugated anti-IgM reagent. Suspensions of spleen and peripheral blood lymphocytes, which were observed but not counted, revealed similar patterns of fluorescence, although some villous lymphocytes did not fluoresce (Fig. 2).

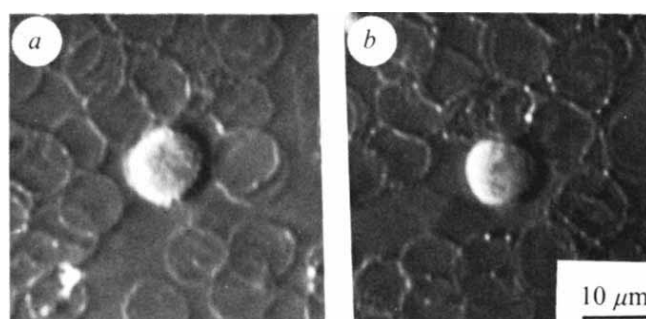


Fig. 3 Peripheral blood smear from C57BL/6 mouse, air-dried and restored. *a*, B lymphocyte with restored surface projections and serrated border; note background of lysed red blood cells. *b*, Smooth surface T lymphocyte with smooth border ($\times 40$ achromat objective, $\times 10$ ocular, Kodak SO 410 35-mm film).

Table 1 Quantitation of lymphocytes with stated appearances as viewed by Hoffman modulation contrast microscopy

Organ cell suspension	Mouse	Smooth cells	Villous cells	% Villous cells
Peripheral blood	BALB/c	431	63	13
	Dublin Swiss	348	52	av. 13 (10–17)
Spleen	C57BL/6	367	133	av. 26.6 (25–30)
	BALB/c	156	44	av. 22 (21–23)
	Dublin Swiss	860	240	av. 22 (18–31)
Thymus	C57BL/6	998	2	0.2
	BALB/c	497	3	0.6
	Dublin Swiss	294	6	2.0
	Dublin Swiss	550*	1*+3†	0.5

*Non-fluorescent. †Fluorescent cells counted by reflected fluorescent and transmitted modulation contrast microscopy.

The characteristic leukocytes found in mouse blood smears were identified by modulation contrast as granulocytes, monocytes and lymphocytes. There was no appreciable loss of adherent leukocytes after a 30-s immersion in physiological buffered saline. Differential counts with standard Wright's stained slides revealed comparable differential counts with duplicate specimens differentiated for surface characteristics by modulation contrast microscopy (Table 2). The lymphocyte population included cells with small surface projections, closely grouped and extending from the peripheral borders, giving a serrated appearance (Fig. 3a), which were designated B cells. Other lymphocytes had smooth peri-

pheral borders and smooth surfaces, including some with one or two flattened projections or folds (Fig. 3b) and they were designated T lymphocytes. Counts of normal lymphocytes in each smear specimen gave a ratio of 89–92% of T cells and 8–11% of B cells (Table 2). The ratio of villous and smooth surface lymphocytes in the peripheral blood suspensions (average values of 13% and 87%) was similar with that of the restored blood smear preparations. This differentiation of T and B cells supports the observations of Rabellino *et al.*⁴. Similar surface differences have been revealed by Nomarski interference contrast on unfixed human¹⁰ and guinea pig⁵ lymphocyte suspensions.

We cannot reconcile our observations with those of Alexander and Wetzel², who found no differentiation of villous and smooth cells among mouse and human lymphocyte populations prepared for scanning electron microscopy. They showed that the smooth lymphocyte population observed by scanning electron microscopy by Polliak *et al.*¹ may have resulted because fixation smoothed the surface structures nonspecifically. Our observations on living cell suspensions indicate no surface changes on smooth or villous surfaces during up to 1 h.

Table 2 Comparison of leukocyte count in Wright-stained and modulation contrast microscopy of blood smears of mice

Mouse	Wright stain			Modulation contrast					
	L	G	M	T	B	%T	%B	G	M
No. 1-BALB/c	85	13	2	80	9	90	10	10	1
No. 2-BALB/c	82	16	2	78	6	92	8	14	2
No. 3-BALB/c	89	10	2	81	8	91	9	10	1
No. 1-C57BL/6	87	10	3	77	9	90	10	12	2
No. 2-C57BL/6	88	11	1	78	9	89	11	10	3
No. 3-C56BL/6	88	10	2	78	10	89	11	10	2

G, Granulocytes; M, monocytes; T, T lymphocytes; B, B lymphocytes.

In all cases the anti-mouse IgM fluorescent stain selected cells with villous projections, whereas if the environment changed the villous cells to smooth cells, the stain would have been expected among the smooth population. Some villous lymphocytes did not fluoresce, perhaps because the IgM antiserum was not specific for those surfaces: another heavy chain gamma globulin fraction of antiserum could be reactive.

Although with restored preparations it was possible to distinguish B and T lymphocytes, villous projections were shorter and less distinct. Conditions for viewing smears are optimal when preparations are restored within 24 h at room temperature. Optimal physical conditions for maintaining dried smears have not been explored fully.

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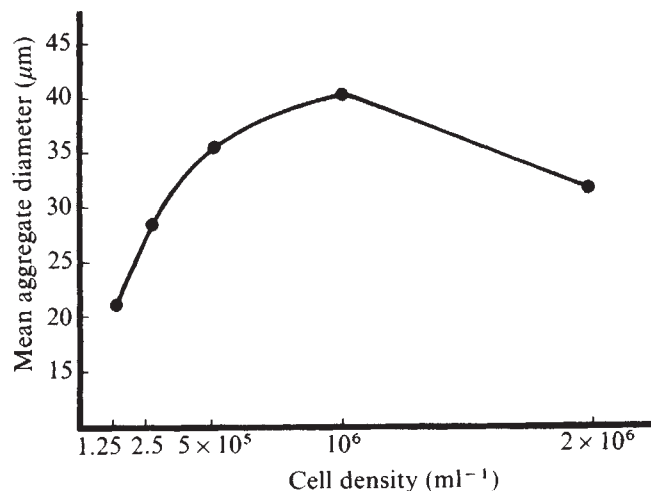
Inhibitory effect of dibutyl cyclic AMP and theophylline on the aggregation of human breast tumour cell line BT-20

DISSOCIATED cells from embryos are able to reaggregate to form multicellular structures resembling those of the original tissue^{1,2}, this ability being inversely proportional to the age of the embryo^{1,3} or to the age of the cells in tissue culture⁴. This intracellular adhesion is tissue specific, and is believed to have a key role in growth and morphological differentiation⁵. It has also been noted that many tumour cell lines can reaggregate in the same condition as embryonic cells^{6–10}. Leighton has observed the formation of small aggregates when tumour cells were grown in cellulose sponge¹¹. He directs attention to the fact that many clinical carcinomas appear as aggregates or nests of cells, and suggests that factors intrinsic to the formation of aggregates are involved in the spread of cancer.

It has been shown that cyclic AMP may influence factors of the cellular membrane such as concanavalin A (con A) agglutination¹², surface antigens on cells¹³, morphology of cells¹⁴, contact inhibition of growth¹⁵, and adhesion of cells to glass^{16,17}. We report here that dibutyl (db) cyclic AMP and theophylline inhibit the aggregation of human mammary tumour cell line BT-20 (ref. 18).

The method of aggregating the cells is essentially that of Moscona¹. BT-20 cells were grown in the medium RPMI 1640, with 10% foetal calf serum and 20 $\mu\text{g ml}^{-1}$ of insulin added. Suspensions of single cells were prepared from confluent cultures by trypsin and EDTA. Aliquots of cell suspensions (3 ml) containing 10^4 – 2×10^6 cells ml^{-1} were placed in 25-ml Erlenmeyer flasks and shaken on a gyrotary shaker for 24 h at 37 °C at 72 r.p.m., with a radius of rotation of 1.25 cm. The cells were washed twice in serum-free medium and then suspended in RPMI 1640 buffered with 20 mM HEPES to give the desired cell density. Serum and insulin were omitted from the medium during experiments, except where the effect of serum was studied. Stock solutions of db cyclic AMP (10 mM), cyclic AMP (10 mM), theophylline

Fig. 1 Effect of cell density on aggregation size of BT-20 cells. Single cell suspensions at 1.25×10^7 to 2×10^6 cells ml^{-1} in RPMI medium without serum were placed in 25-ml Erlenmeyer flasks in 3-ml aliquots. The cell suspensions were incubated in a gyrotary shaker at 72 r.p.m. for 24 h. Samples (0.5 ml) were removed from each flask and placed in a plastic Petri dish. Photomicrographs of the aggregates were taken and the diameter of the aggregates measured. The mean size of aggregates from four flasks is shown here.



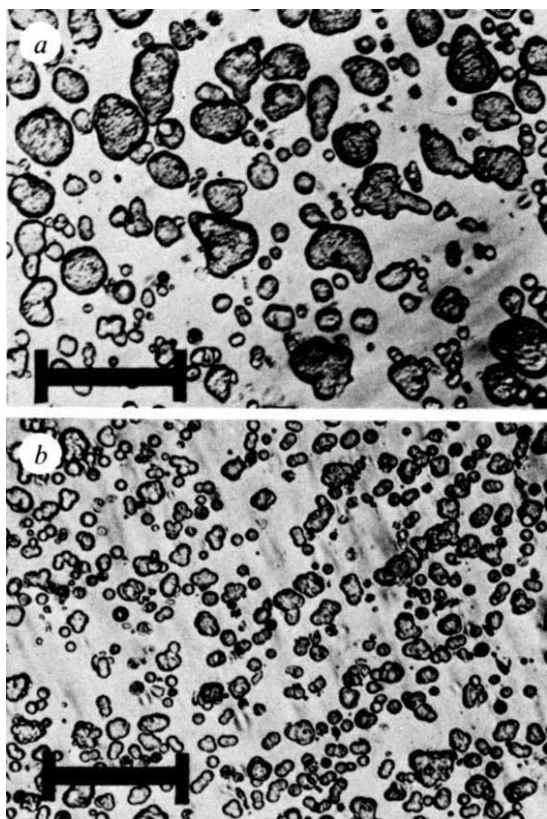


Fig. 2 Photomicrographs of aggregates made from a suspension of cell density 0.5×10^6 cells ml^{-1} . *a*, Control aggregates; *b*, in the presence of 1 mM db cyclic AMP and 1 mM theophylline. Bar, 200 μm .

(10 mM) or papaverine (1 mM) were each made up in RPMI 1640. The size of the aggregates was measured by placing 0.5 ml of the suspension in a 35-mm plastic Petri dish, and photographs of the aggregates taken. The negative was then projected through an enlarger at a fixed distance. The outlines of the aggregates were traced on to paper and the size of each aggregate measured by comparing with standard metric circle and ellipse templates. The results were then expressed in μm diameter of cross section of the aggregates. About 300 aggregates were analysed for each determination.

The aggregation of BT-20 over a 24-h period depended on the number of cells present in the incubation. Figure 1 shows that the mean size of aggregates obtained was maximum at about 0.5×10^6 – 1.0×10^6 cells ml^{-1} . A 0.5 ml volume of a suspension of this cell density yielded a reasonable number of discrete aggregates for measurement. In the presence of 10% serum the aggregates were slightly larger, but many more cells adhered to glass. Occasionally a thick cord of cells detached from the glass making measurements difficult. Using a standard density of 5×10^5 – 10^6 cells ml^{-1} , we found that the addition of 2 mM db cyclic AMP and 2 mM theophylline greatly inhibited the aggregation of BT-20 cells. Figure 2*a* is a photomicrograph of the control aggregates, and Fig. 2*b* shows aggregates obtained in the presence of db cyclic AMP and theophylline. In Fig. 3*a* we present histograms of aggregate size in the control and treated cultures. The figures show that db cyclic AMP and theophylline restricted the diameters of aggregates to less than 25 μm . In Table 1 we show the percentage of aggregates with diameters $\geq 25 \mu\text{m}$ in the control and treated incubations. After 24 h, db cyclic AMP and theophylline inhibited reaggregation by a factor greater than 4.

The inhibitory effect of db cyclic AMP and theophylline was reversible. After removal of the drugs, the cells and aggregates were washed three times with 10 ml of medium, resuspended in 3 ml of medium, and then incubated on the gyrotary shaker. Comparison of Fig. 3*b* (48-h aggregates) with Fig. 3*a* (24-h aggregates) shows that after the further 24-h incubation there were many more large aggregates and the distribution of the size of aggregates was comparable with that of the control at 24 h. The control aggregates after 48 h of incubation also increased in size as shown in Fig. 3*b*. In another experiment, similar results were obtained when aggregates from treated incubations were washed and trypsinised to yield suspensions of single cells. These cells reaggregated to the same extent as the control cells.

To ascertain that db cyclic AMP and theophylline did not kill BT-20 cells, we carried out the Trypan blue exclusion test after 24 h of incubation. Both treated and control incubations showed the same degree of cell viability ($>90\%$). Single cells and aggregates in treated incubations also attached to glass and spread out after removal of the drugs and reincubation without shaking. The inhibitory effect of db cyclic AMP and theophylline was dependent on concentration. Figure 4 shows the increasing degree of inhibition with increasing concentration of drugs. It is possible that db cyclic AMP was hydrolysed by the cells to butyrate, which has been shown to produce a similar effect on cells as cyclic AMP¹⁹. We tested the effect of sodium butyrate on BT-20 aggregation, and found that 2 mM butyrate was highly toxic to cells. At a concentration of 0.5 mM, however, toxicity was not detected and there was no effect on cell aggregation. The compound cyclic AMP alone also inhibited aggregation, but a much higher concentration (5–10 mM) was necessary.

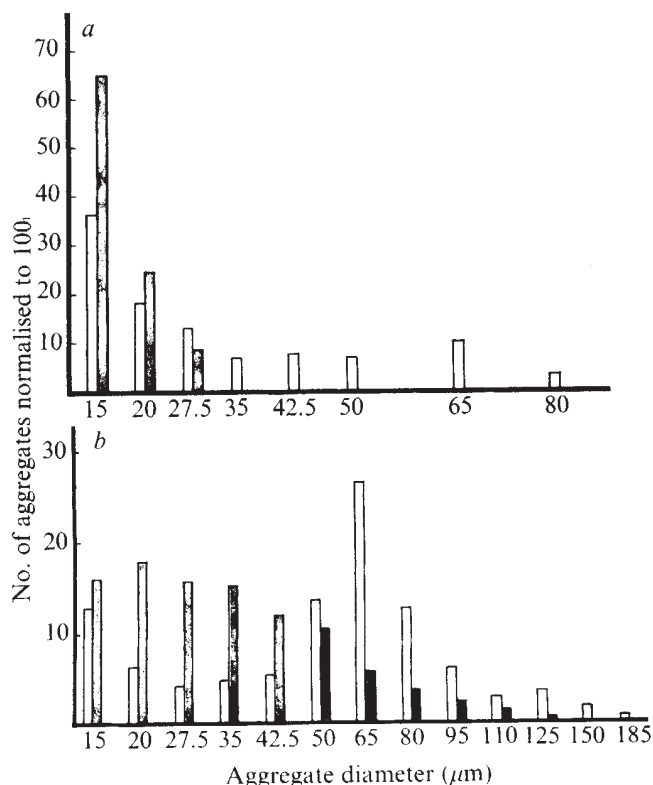


Fig. 3 Histograms showing the effect of db cyclic AMP and theophylline on aggregation of BT-20 cells. *a*, 24-h incubation. Open bars, control aggregates; shaded bars, aggregates treated with db cyclic AMP and theophylline. *b*, 48-h incubation. After the first 24-h incubation the aggregates were collected, spun down, washed, and resuspended in RPMI medium without serum or drugs for the second 24-h incubation. Open bars, control aggregates; shaded bars, aggregates treated with db cyclic AMP and theophylline for 24 h and recovery after removal of the drugs.

Table 1 Inhibitory effect of db cyclic AMP and theophylline on aggregation of BT-20 cells

	Control	db cyclic AMP + theophylline	Control	Recovery*
	24 h	24 h	48 h	48 h
Aggregates with diameters ≥ 25 µm	46.2	10.7	80.6	66.1

Results are expressed as the percentage of aggregates greater or equal to 25 µm diameter in relation to the total number of aggregates measured.

*After treatment for 24 h with db cyclic AMP and theophylline, the cells were centrifuged, washed three times with 10 ml of medium, and then resuspended in 3 ml of medium. The aggregates were measured 24 h later.

The compound db cyclic AMP has been shown to be more resistant than cyclic AMP to hydrolysis by phosphodiesterase²⁰. We have further tested the effect of theophylline alone on reaggregation, and found that it was almost as effective as the combination of db cyclic AMP and theophylline. Of the other phosphodiesterase inhibitors, we have tested papaverine and trifluoperazine. The latter was very toxic to BT-20 cells at 50 µM. At 20 µM, however, toxicity was not apparent and there was no effect on aggregation. Papaverine, on the other hand, was well tolerated by BT-20 cells. At 100 µM it produced 70–80% inhibition of aggregation without toxicity. (A detailed report on the effect of papaverine on cell–glass and cell–cell adhesion is in preparation.)

The phenomenon of cell aggregation may involve several steps. Orr and Roseman²¹ have found that the initial intercellular adhesion of embryonic chick neural retina cells was not affected by cyclic AMP, but Kuroda²² found that cyclic AMP during 24 h of incubation inhibited the aggregation of embryonic neural retina cells. We have shown in this series of experiments that cyclic AMP and some inhibitors of phosphodiesterase inhibited aggregation of BT-20 cells in suspension. A number of investigators^{23–25} have observed that cellular junctions, such as desmosomes, form in aggregates. Some preliminary electron microscopic studies on BT-20 aggregates also showed the formation of desmosomes after 24 h of incubation. We are pursuing the investigation to see if cyclic AMP inhibits the formation of desmosomes.

Several studies^{10, 26–29} indicate that aggregation-promoting factors of a glycoprotein nature are involved in a number of systems from sponge cells to rat hepatoma cells. Since

cyclic AMP can influence a number of cell surface activities, it is conceivable that its inhibitory effect on cell aggregation is due to a change in the orientation of these 'aggregation molecules' by an alteration of membrane fluidity, or a direct effect on the biosynthesis of these molecules.

The inhibitory effect of cyclic AMP on membrane activities of human cells has also been noted by Bryant and Sutcliffe³⁰, who found that high concentrations of intracellular cyclic AMP inhibited the adhesiveness of human basophils, eosinophils, and neutrophils. Increasing the concentration of cyclic AMP level also inhibits human platelet aggregation³¹.

Our experiments suggest that cyclic AMP, by its modulation of adhesion between carcinoma cells as they form aggregates, may have a role in the propagation of cancer³².

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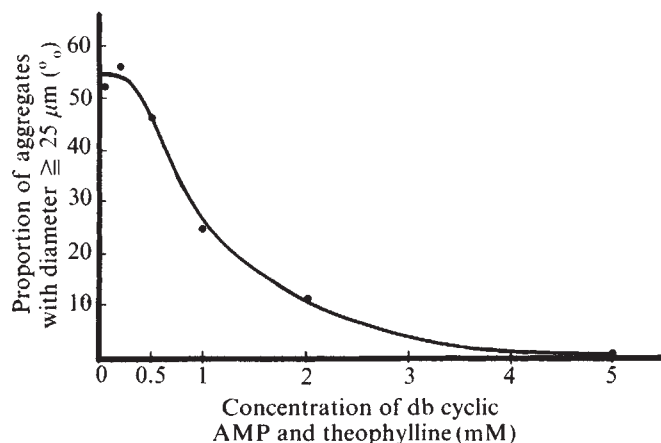
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Fig. 4 Effect of varying concentrations of db cyclic AMP and theophylline on aggregation of BT-20 cells, which were incubated in 3 ml of medium on a gyrotary shaker. The medium contains db cyclic AMP and theophylline at various concentrations. The sizes of aggregates at 24 h were measured.



Abortogenic activity of antiserum to alpha-fetoprotein

ALPHA-FOETOPROTEIN (AFP), a glycoprotein of molecular weight 70–72,000, is synthesised by the yolk sac and by perivascular parenchymal cells of the mammalian foetal liver and gastrointestinal tract^{1–3}. Increased levels of AFP occur in maternal and foetal sera during pregnancy^{4,5}, and indirect evidence suggests some production of AFP by the chorionic tissues⁶. Increase of AFP in amniotic fluid has been correlated with neural tube defects, anencephaly, hydrocephaly, congenital nephrosis and foetal death^{7–10}. The specific function of AFP has not been determined, but oestrogen-binding and immunoregulation during pregnancy have been implicated^{11,12}. Smith¹³ noted an effect of rabbit antiserum to rat AFP when injected into pregnant rats. We now report evidence for a specific abortogenic activity

of rabbit antiserum to murine AFP (anti-AFP) when administered during gestation in the laboratory mouse.

Approximately 100 pregnant mice (day 9–20 of gestation) of the DB2A, C57BL, or Nya:NYLAR strains were injected intraperitoneally with 0.5–2.0 ml of specific anti-AFP (prepared as described under Table 1), with non-immune rabbit serum (NRS), or with buffered saline. The mice were examined 6, 12, 18 and 24 h after injection, and the cages were inspected for aborted fetuses. At 24 h, the mothers were autopsied; the litter size and foetal dimensions were determined. Foetuses and neonates from mothers in all treatment groups were examined for gross morphological changes. Histological sections of the foetal and maternal tissues were prepared using Bouin's fixation and haematoxylin and eosin. The uteri with implantation sites were examined in multiple sections. Samples of maternal liver, ovary, lung, spleen and kidney were examined routinely.

Three levels of response were noted: no effect; partial abortion indicated by subplacental haemorrhage; and complete abortion. Passage of aborted fetuses usually occurred 16–20 h after injection. Anaphylaxis was rarely observed. Of the animals treated with specific anti-AFP, 68% exhibited a physiological response (Table 1). Partial and complete abortions both occurred in the latter two thirds of gestation. Partial abortions tended to occur earlier and complete abortions slightly later. As a rule, the greater the

foetal mass, the more anti-AFP was needed to produce the response. During mid-pregnancy 0.5–1.0 ml of intra-peritoneally administered anti-AFP was sufficient to produce abortion. Towards the end of pregnancy, induction of abortion was less frequently successful and required up to 2.0 ml of anti-AFP.

Gitlin and Koch¹⁷ showed that heterologous IgG readily passes through the placenta in the mouse by both first and second order reactions. Thus, the passage of rabbit anti-AFP antibodies across the mouse placenta is likely. In the pregnant rat, Sell *et al.*¹⁸ and Colquhoun *et al.*¹⁹ reported a steep increase of AFP in maternal serum beginning at days 10–14 and continuing to birth. Sell and Alexander²⁰ further demonstrated that a catabolic site for AFP is present in the foetus or placenta during the last week of gestation in the rat. These studies indicated that increasing amounts of AFP traverse the placenta as development proceeds. A similar increase of AFP levels in late pregnancy in mice could account for a neutralisation of anti-AFP antibodies at the doses we tested.

The difference in response levels of the three treatment groups was significant by χ^2 analysis ($P < 1.0\%$). None of the animals injected with buffered saline exhibited physiological or pathological effects. More than 90% of the mice treated with NRS also showed no effect. In the three animals aborted by NRS, the effect occurred on days 15 and 16. This suggested a nonspecific susceptibility of the uterus to extrinsic factors at this time.

To confirm the specificity of the anti-AFP effect, we tested rabbit antisera to whole mouse serum and to three mouse protein antigens that are similar to AFP in either structure or site of origin: albumin, complement (C3) and transferrin²¹. These antisera caused neither partial nor complete abortion in any animal tested (Table 1). As a further test of specificity, aliquots of anti-AFP for injection were absorbed individually with freshly prepared pregnant uterine tissue extracts or with purified AFP fractions obtained from column fractionation. The tissue saline extracts were prepared according to Witebsky's²² method. Serum specificity was tested by blocking experiments, using both purified AFP antigen and tissue (uterus and spleen) absorption. The tissues from six pregnant mice (13–18 d gestation) were homogenised in phosphate-buffered saline (pH 7.6) and the resultant tissue brei was absorbed in equal proportions (w/v) with the antisera for 30 min at 37 °C, then overnight at 4 °C. Precipitation-inhibition results were confirmed by immunodiffusion. With two exceptions the absorbed antisera produced no effect when injected into pregnant mice at days 13–16 of gestation. Anti-AFP absorbed with a murine spleen tissue extract retained abortogenic activity, and anti-AFP absorbed with one of three uterine tissue samples caused an anaphylactic response.

The mechanism of pregnancy interruption by anti-AFP is unknown, although the specificity of the reaction is evidenced by target tissue absorption and by the lack of effect of rabbit antisera to other mouse serum proteins. Except for intrauterine clot formation, histopathological studies were unrevealing. There seemed to be no inflammatory or lymphocytic infiltrates in the decidua or placenta, and vasculitis was not observed in any tissue. Although studies by Smith¹³ demonstrated that the injection of pregnant rats with anti-AFP serum may also result in developmental abnormalities, none was observed in autopsied or surviving litters of any of the mice we treated.

The abortogenic activity of anti-AFP may be due to an anaphylactoid contraction of the uterine smooth muscle; that is, the response may be an *in vivo* correlate of the Schultz-Dale reaction²³. This humoral mechanism would require the presence of AFP in the uterine or placental tissues. The demonstration by Michel *et al.*²⁴ of AFP receptors in the immature rat uterus provides some support for this hypothesis.

Table 1 Response of pregnant mice to intraperitoneally injected rabbit anti-AFP, non-immune rabbit sera (NRS), physiological saline or rabbit antisera to other mouse serum proteins

Treatment group	Response	No. Gestational age (d)*		
		of animals	Range	Mode
Anti-AFP	Complete abortion	11/31	12–17	15
	Partial abortion	10/31	9–14	13
	No effect	10/31	14–20	18
NRS	Complete abortion	3/36	15–16	15
	No effect	33/36	9–20	15
Saline	No effect	16/16	9–20	17
Anti-transferrin	No effect	4/4	15–18	17
Anti-complement	No effect	4/4	14–17	15
Anti-albumin	No effect	6/6	12–15	15
Anti-whole mouse sera	No effect	6/6	11–18	14

Rabbit antiserum to murine AFP (anti-AFP) was produced by immunisation with antigen fractions derived from amniotic fluid¹⁴. The AFP was isolated by gel filtration on Sephadex G-75, followed by shallow-gradient DEAE ion exchange chromatography and preparative gel electrophoresis¹⁵. Disc gel electrophoresis showed a single band, and after immunoelectrophoresis a single precipitin arc was present in the α -1 region. In spite of these indications of purity, albumin was detected as a trace contaminant after immunisation and was eliminated by absorption using two parts normal mouse sera to eight parts antiserum. The absorbed anti-AFP was immunoreactive to mouse AFP as evidenced by single-band formation after immunodiffusion and counterimmunoelectrophoresis. Anti-AFP from four rabbits were used. These antisera had specific antibody concentrations of 0.424–0.532 mg ml⁻¹ as determined by the quantitative precipitin reaction. Each anti-AFP serum was tested at all stages of pregnancy in separate experiments. The results included in Table 1 summarise all these data. Control sera consisted of non-immune rabbit serum (NRS), rabbit anti-whole mouse sera, rabbit anti-mouse albumin, rabbit anti-mouse transferrin and rabbit anti-mouse complement. The NRS serum was absorbed with mouse sera as described above. The anti-albumin serum was prepared from mouse albumin isolated by ion exchange chromatography; the antisera to whole mouse sera, transferrin and complement were obtained from Microbiological Associates. The specificity of these control sera was confirmed both by immunoelectrophoresis and immunodiffusion using whole and fractionated mouse serum. The immunological activity of each of the four anti-AFP and of the control antisera was attributed to the IgG, as determined by immunoelectrophoresis. The anti-AFP and anti-albumin sera were obtained from hyper-immunised rabbits bled 6 weeks after the first injection thus assuring a predominance of IgG antibody in the serum.

*Length of gestation at time of injection. Gestational age was estimated by palpation, and foetal size was determined by crown-rump measurements according to Rugh¹⁶.

An alternative possibility is suggested by the work of Murgita and Tomasi^{22,25}, who demonstrated that AFP suppresses both the primary and secondary antibody response to sheep red blood cells without inducing cytotoxic effects *in vitro*. Further studies by Murgita and Tomasi revealed that AFP can suppress allogeneic and mitogen-induced lymphocyte transformations (T-cell dependent functions) in mice²⁵. Finally, immunofluorescence studies by Dattwyler *et al.*²⁶ strongly suggest the presence of AFP receptors on the surface of certain T-cell lymphocyte populations in mice. On the basis of these findings, it can be postulated that AFP is instrumental in maintaining pregnancy, the foetus being viewed as an allograft in the maternal tissues. Extrapolating this hypothesis, neutralisation of AFP at the maternal-placental interface might conceivably initiate rejection of the foetus through a cell-mediated immune mechanism. Although evidence of a graft (foetus) rejection was not histologically apparent, the physiological release by lymphocytes of vasoactive lymphokines, such as prostaglandins²⁷, and/or the release of T-cell suppressor function might not necessarily leave a morphological trace.

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Early production of intracellular IgM by B-lymphocyte precursors in mouse

IN BALB/c mice, surface immunoglobulin (Ig)-bearing B lymphocytes are first detectable by immunofluorescence at 17 d gestation in the liver and spleen¹. Explants of 14-d foetal liver¹ and spleen², and 15-d bone marrow (our unpublished observations), have been shown to generate Ig-bearing B cells *in vitro* after 4-7 d of culture, suggesting that B lymphocytes normally develop multifocally in the haemopoietic tissues of mice. We have used direct immunofluorescence to demonstrate cells with intracellular IgM and no detectable surface Ig in mouse foetal liver as early as 12 d gestation. Our results suggest that B lymphocyte precursors synthesise IgM several days before they incorporate these molecules into their plasma membranes as cell-surface receptors for antigen.

Purified goat antibodies to mouse κ chains (anti- κ), μ chains (anti- μ), α chains (anti- α) and $\gamma 2a$ and $\gamma 2b$ chains (anti- $\gamma 2$) were raised, adsorbed and purified on various immunoadsor-

bants, and tested for specificity, as previously described³⁻⁵. While the anti- $\gamma 2$ antibodies used in our original experiments on the development of B cells in foetal liver explants¹ were subsequently found to have contaminating antibodies against κ chains (probably accounting for most, and possibly all, of the apparent $\gamma 2$ -bearing cells seen), the anti- $\gamma 2$ (as well as the anti- μ and anti- α) antibodies used in the experiments reported here could not be demonstrated to have any anti- κ activity, and were class specific by Ouchterlony analysis, immunofluorescence staining of fixed cell preparations of various mouse myelomas, and modulation experiments in which each antibody preparation did not modulate surface Ig of other classes at a concentration of 1 mg ml⁻¹. The purified antibodies were conjugated to fluorescein or rhodamine as previously described³⁻⁴. In some experiments purified polyspecific goat anti-mouse Ig conjugated to fluorescein (anti-MIg-FI) was used⁶. Nonspecific staining of fixed cells was eliminated by adsorbing the conjugates with mouse liver powder. The conjugated antibodies were used at a final concentration of 500 μ g ml⁻¹.

Table 1 Ontogeny of IgM⁺ cells in BALB/c foetal liver*

Gestation (d)	Surface IgM ⁺ cells (%)†	Intracellular IgM ⁺ /surface IgM ⁻ cells (%)‡
12	0, 0	0, 0.03
13	0, 0, 0, 0, 0, 0	0.015, 0, 0.007, 0, 0.03, 0
14	0, 0, 0, 0	0.33, 0.05, 0, 0.05
15	0, 0, 0	0.02, 0.1, 0.06
16	0, 0	0.32, 0.2
17	0.15, 0.10	0.75, 2.0
18	0.6	2.8
19	1.1	4.2

*Cells were stained in suspension with anti- μ -Rd to label surface IgM and with anti- μ -FI (after cyto centrifugation and fixation) to label intracellular IgM. As discussed in the text, surface IgM⁺ cells stained with both rhodamine and fluorescein conjugates while intracellular IgM⁺/surface IgM⁻ cells stained only with fluorescein. Usually, more than 50,000 cells were scanned.

†Each number represents one experiment.

‡These cells also failed to show surface labelling with polyspecific anti-MIg, suggesting the lack of any detectable surface Ig.

Haemopoietic and lymphoid tissues were removed from BALB/c embryos of various ages (the day of appearance of a vaginal plug being taken as day 0), newborns or 6-week-old adults, and dissociated into single cell suspension in Dulbecco's modified Eagle's medium containing 15 mM HEPES, 0.2% sodium azide and 10% foetal calf serum (DMEM). The cells were labelled in suspension with rhodamine-conjugated anti-Ig antibodies at 0 °C for 30 min to demonstrate surface Ig, washed and cyto centrifuged onto a glass slide, fixed in cold 5% acetic acid in ethanol, and stained for intracellular IgM with fluorescein-conjugated anti-Ig antibodies at 37 °C for 25 min. After thorough washing, the cells were examined under a coverslip for surface (rhodamine) and intracellular (fluorescein) fluorescence, using a Zeiss Ultraphot II, or a Leitz Ortholux microscope, equipped with phase contrast and incidence fluorescence optics and an Osram HBO 200 mercury arc lamp. Fluorescein-conjugated anti-Ig antibodies for surface staining and rhodamine conjugates for intracellular staining gave the same results.

As has previously been reported¹, cells in foetal liver with detectable IgM on their surface were first seen at 17 d gestation (Table 1). But foetal liver cells with readily detectable intracellular IgM and without detectable surface Ig were first seen at 12 d in some mice and by 15 d in all litters studied (Fig. 1). Although most of these cells were large (comparable in size with medium or large lymphocytes), some were the size of small lymphocytes. The staining of fixed cells with fluoresceinated-anti- κ antibodies (anti- κ -FI) gave similar results to those obtained with anti- μ -FI, but no intracellular labelling was seen with anti- $\gamma 2$ -FI or anti- α -FI. This attested to the specificity of the intracellular labelling with anti- μ -FI and anti- κ -FI, and indicated that these intracellular IgM⁺/surface Ig⁻ cells

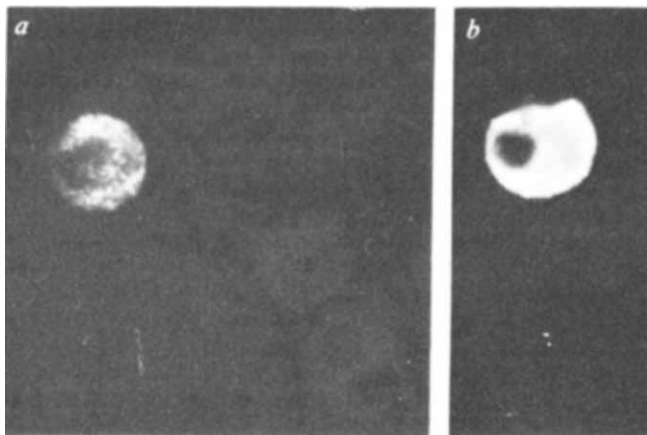


Fig. 1 *a*, Cell stained for intracellular IgM with anti- μ -Rd in 15-d foetal liver; *b*, For comparison, a plasma cell in adult spleen stained for intracellular IgM with anti- μ -Rd. The film was exposed for 3.5 min in *a* and 2 s in *b* ($\times 300$).

produced both heavy (μ) and light Ig chains. Nonspecific labelling due to binding to Fc receptors was also excluded by showing that $F(ab')_2$ fragments of anti- μ antibodies, conjugated to fluorescein, gave results similar to those obtained with undigested anti- μ -Fl.

To detect intracellular Ig, cells must be fixed before labelling, while cell surface Ig can be demonstrated by labelling either living cells in suspension or cells fixed on a slide. Thus, cells with surface Ig (B cells) stained with both fluorescein and rhodamine in our double labelling technique, whether or not they had detectable intracellular Ig. Therefore, to determine whether these cells also had detectable intracellular Ig, we labelled surface IgM on living adult spleen cells with anti- μ -Rd in capping conditions⁷ (DMEM without azide for 20 min at 20 °C) and then looked for intracellular IgM with anti- μ -Fl, after fixation. All the fluorescein labelling coincided with the rhodamine labelling in the caps and was not seen elsewhere in the cell, indicating that most surface-IgM⁺ cells in spleen did not have intracellular IgM detectable by this means. In a similar experiment with newborn liver cells, a small proportion of the surface IgM⁺ cells also showed intracellular IgM.

As Table 2 shows, intracellular IgM⁺/surface Ig⁺ cells were found only at sites of B lymphocyte production, such as foetal and newborn liver¹ and spleen² and adult bone marrow^{7,9}. They were not found in adult lymph node or spleen, or in foetal thymus. Their distribution, and the fact that they synthesise Ig, strongly suggest that they are B lymphocyte precursors, and we will refer to them subsequently as pre-B cells. IgM-containing plasma cells and other IgM-secreting lymphoid cells in adult lymphoid tissues, were readily distinguished from pre-B cells because the former stained much more intensely with anti- μ -Fl (Fig. 1) and always had readily detectable IgM on their surface. Table 2 shows that most IgM-synthesising cells in foetal and newborn liver and spleen, and in adult bone marrow are pre-B cells rather than B cells. Since they appear several days earlier in ontogeny than do B cells with surface Ig, one should be able to use fluorochrome-labelled antigens to study the generation

of antibody diversity earlier than previously possible. In addition, having an intracellular marker for pre-B cells should be useful for studying B-cell development in normal and immunodeficient animals, including man.

The pre-B cells in adult bone marrow described here presumably are the same as, or overlap considerably with, the surface Ig⁺ cells, previously described in bone marrow, which can differentiate into surface Ig⁺ B cells *in vitro* and *in vivo*^{8,9}. Our failure to detect significant numbers of pre-B cells in the adult spleen suggests that this organ is not an important site where pre-B cells differentiate to B cells in adult animals, and that most "null cells" (cells lacking T or B cell surface markers)^{6,10} in the spleen probably do not belong to the B-cell lineage. It also seems likely that our pre-B cells in foetal liver are among the "Type 1 B cells" described by Melchers *et al.*¹¹ as large cells in 10–12-d mouse embryos and in 13–15-d foetal liver and in adult bone marrow, which incorporate radioactive leucine into rapidly turning over ($T_{1/2}$ = 1–3 h) 7–8S IgM. Since such 7–8S IgM could be labelled by lactoperoxidase-catalysed radioiodination of adult bone marrow cells, Melchers *et al.* suggested that it is on the cell surface, and that the failure of others to demonstrate it there may be due to its rapid turnover¹¹. This is an important question, as the presence or absence of Ig at the cell surface would determine whether or not such cells could bind, and possibly respond to antigen.

Table 3 IgM⁺ cells in foetal liver explants inhibited with anti- μ antibody

Age of liver (d)	Days in culture	Anti- μ in culture (μ g ml ⁻¹)	Surface IgM ⁺ cells (%)	Intracellular IgM ⁺ /surface IgM ⁺ cells (%)
18	3	0	1.8	2.9
18	3	20	0	3.2
14	7	0	7.4	9.5
14	7	20	0	7.2

To help resolve this apparent discrepancy, we cultured foetal liver fragments on top of Millipore filters as described previously¹ in the presence or absence of unconjugated anti- μ antibodies (20 μ g ml⁻¹). We have shown previously that anti- μ antibodies in such cultures induce the disappearance (and prevent the development) of cell-surface IgM⁵. As Table 3 shows, although no surface IgM was found on anti- μ treated cells this treatment did not affect the proportion of cells with intracellular IgM or the intensity of their staining. Thus most of the IgM that we have detected as intracellular in pre-B cells by our double labelling technique seems not to be available at the cell surface, and unlike B cells⁵, these cells are not killed or suppressed by prolonged exposure to anti-Ig antibodies. These experiments do not exclude the possibility that a small proportion of the IgM synthesised by these cells is exposed at the cell surface.

The biological significance of the intracellular IgM in pre-B cells is enigmatic. It seems more likely that it is destined for the plasma membrane than for secretion, but then it is not clear why there is such a long period between synthesis and incorporation into the cell surface. It may be that it provides a mechanism for protecting these cells from interacting with antigen during a period of clonal expansion and/or diversification of activated V genes. If the IgM is destined for the plasma membrane, the failure to find detectable intracellular IgM in B cells with surface IgM, suggests that it may accumulate in pre-B cells because it cannot get to, or be stably incorporated into, the plasma membrane. Perhaps, this incorporation must await the production of an appropriate glycosyl-transferase, proteolytic enzyme, or cell-surface acceptor protein. Whatever the explanation, these cells may provide a useful system for studying where membrane proteins are made and how they are distributed to their appropriate location in the cell.

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Table 2 Distribution of IgM⁺ cells in various BALB/c tissues

Tissue	Age	Surface IgM ⁺ Cells (%)	Intracellular IgM ⁺ /surface IgM ⁺ cells (%)
Foetal thymus	14 or 17 d	<0.01	<0.01
Spleen	Newborn	3.9	4.9
Spleen	6 weeks	31	<0.1
Lymph nodes	6 weeks	18	<0.1
Bone marrow	6 weeks	7	12
Liver	Newborn	1.4	3.3

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Resistance of activated macrophages to H-2 antibody-mediated cytotoxicity and Fc rosette inhibition

MACROPHAGES can have critical roles in repair, inflammation and a large variety of immunological phenomena^{1,2}. The biological roles of these cells often occur subsequent to their 'activation', a subjective term which refers to a greatly heightened state of reactivity¹⁻³. Activation can be induced by a variety of stimuli including ingestion of certain macro-organisms, contact with immune complexes, bacterial lipopolysaccharides and lymphokines³. Activated macrophages usually show an increase in cell size, increased adherence properties and cellular processes, lysosomal enzyme activity, and phagocytic ability^{1,2}. In addition, alteration in characteristics and functions of various macrophage receptors can apparently take place as a consequence of activation^{4,5}. Such a change is described in this paper: it involves Fc receptors as detected by Fc or EA rosette formation (see Table 1), and their relationship to surface mouse major histocompatibility (H-2) antigens as detected by anti-H-2 alloantisera. Aside from the general relevance this change has with regard to membrane alterations which occur in activated lymphoreticular cells, it can also be exploited as a marker for activated macrophages.

Recent results have shown that formation of Fc (EA) rosettes between IgG antibody-coated erythrocytes and lymphoid cells bearing Fc receptors can be totally blocked by preincubation of the lymphoid cells with relevant antisera against the major histocompatibility antigen complex^{6,7}. An example of this is seen in Table 1: preincubation of DBA/2 (H-2^d) mouse strain spleen cells with anti-H-2^d alloantiserum resulted in a marked reduction of the ability of the cells to subsequently form Fc rosettes with IgG antibody-coated sheep erythrocytes (SRBCs) compared with normal mouse serum (NMS)-treated controls. Preincubation with irrelevant anti-H-2 serum (for example anti-H-2^b) resulted in no inhibition. When normal peritoneal exudate cells (PECs), containing an average of about 24% macrophages (assessed by ingestion of latex particles) were

similarly tested, rather drastic inhibition in the ability of the cells to form Fc rosettes was once again noted. Since macrophages bear the Fc receptor^{1,4}, these results show that the Fc rosetting ability of most or all normal (unstimulated) macrophages is inhibited by the anti-H-2 pretreatment.

Quite different results were obtained, however, when PECs from BCG-injected or proteose-peptone-injected mice were tested; such injections result in 'activation' of the macrophage population by a variety of criteria^{2,8}, and, as shown in Table 1, such activated PEC populations retained a high degree of their Fc rosetting ability after anti-H-2 serum pretreatment. Equally significant, the proportions of phagocytic cells and anti-H-2-resistant Fc rosettes in these cell suspensions were almost equal (Table 1). It thus seems that it is the activated macrophages which are resistant to the inhibiting effects of the anti-H-2 serum. This was verified by testing plastic-adherent (macrophage-rich) compared with non-adherent (lymphocyte-rich) cells from these cell suspensions: as shown in Table 1, the adherent cells were only minimally inhibited, whereas non-adherent cells were quite severely impaired in their ability to form Fc rosettes.

Activated (blast) lymphocytes, either B cells mitogenically transformed with *Escherichia coli* lipopolysaccharide⁹ (LPS) or T cells activated to allogeneic antigens *in vivo*,

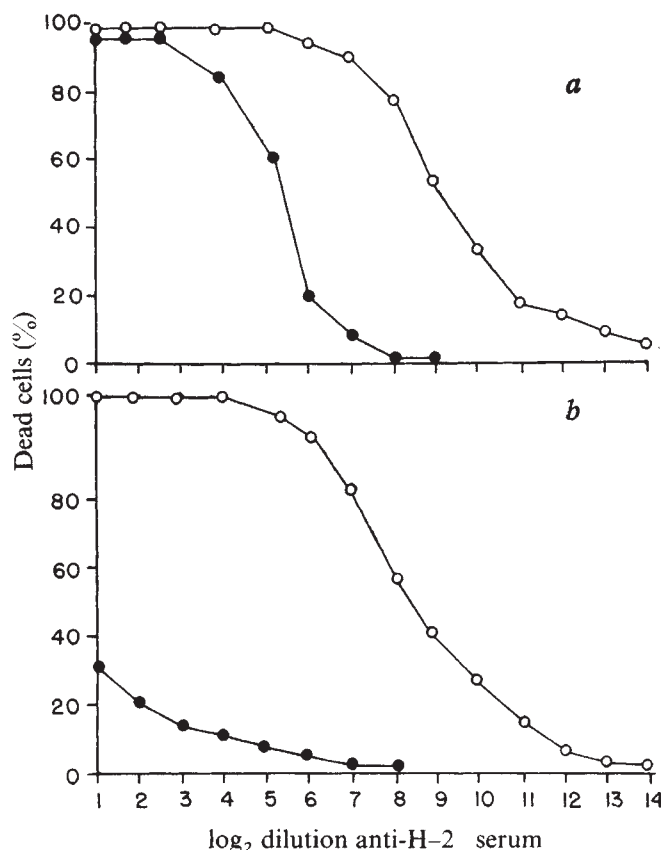


Fig. 1 Cytotoxicity of CBA anti-DBA/2 (anti-H-2^d) serum on DBA/2 spleen (a) or peritoneal exudate cells (PEC) from peptone-stimulated mice (b), using GPC or RC as sources of complement. Dead cells were assessed by Trypan blue uptake²⁰. The PEC population contained about 65% phagocytic cells before testing. The GPC (●) and RC (○) were both purchased from Gibco in a lyophilised form. The GPC was used at a dilution of 1:6. The RC was first extensively absorbed with mouse RBCs, spleen, thymus and various mouse tumour culture cell lines to remove naturally occurring antibodies against mouse nucleated cells. It was then used at a dilution of 1:2. The cytotoxic curve of anti-H-2^d serum on normal PECs was very similar to that found with spleen. These experiments were repeated many times using different sources of GPC, including fresh guinea pig serum, and different batches of anti-H-2^d serum, the same results being obtained.

Table 1 Effect of anti-H-2 serum preincubation on the ability of various cell populations to form Fc rosettes*

Cells tested	% Phagocytic cells	% Fc rosettes obtained after NMS	% Fc rosettes obtained after Anti-H-2 ^d
Spleen	4.4±2.1	47.6±6.0	2.8±1.5
Normal PECs	24.4±8.3	54.0±9.7	3.1±1.6
BCG-activated PECs	31.0±19.6	64.2±15.4	27.0±14.7
Peptone-activated PECs	44.1±12.9	83.3±4.6	47.0±8.2
Adherent activated PECs	79.8±6.6	89.7±5.8	82.5±5.8
Non-adherent activated PECs	5.1±2.0	36.3±8.4	4.3±2.8
GvH spleen†	3.2±0.95	46.0±6.2	3.0±1.1
LPS spleen cells‡	3.5±2.1	44.2±8.9	2.0±p.8
P815 mastocytoma § cell line (H-2 ^d)	0	67.2±35.9	0
L1210 myeloid leukaemia cell line (H-2 ^d)	0	42.7±14.4	0
FVL erythroleukaemia cell line (H-2 ^d)	0	33.5±16.2	0
P388D ₁ macrophage-like cell line (H-2 ^d)	61.5±15.6	95.2±2.0	84.2±11.8
SaD/2 solid tumour ¶	10.8±4.6	25.0±8.2	12.5±4.2
SaD/2 solid tumour (iron-treated)	1.1	14.0	1.8

*Fc rosettes were assessed using SRBCs coated with a 1/100 (subagglutinating) dilution of a heat-inactivated rabbit hyperimmune anti-SRBC serum, as described previously¹². Also previously described¹²: the Fc rosette assay, preparation and properties of the anti-H-2^d (CBA anti-DBA/2) serum, and preparation of the various DBA/2 strain cell suspensions. The inhibitory effect of anti-H-2^d serum was assessed by preincubating 0.5×10^6 cells, previously pelleted by centrifugation with 0.1 ml anti-H-2^d serum or NMS (normal CBA mouse serum) for 20 min, centrifugation of the cells followed by removal of the serum and addition of 0.5 ml of a 1% suspension of the antibody-coated SRBCs. Rosettes were then made and counted using crystal violet to stain the rosetted cells¹². Phagocytic cells were assessed by ingestion of latex beads²² or the osmotic lysis technique^{12,23}. The latex method was found to be more reliable when assessing normal macrophages. Selection of adherent and non-adherent PECs was as described previously¹². The results recorded represent the mean of 4–6 experiments ± standard deviation.

†B cells were activated in culture by the mitogen *E. coli* LPS⁸ as described previously²⁴ and were collected after 3 d. Such cultures contained about 50% 'blast' cells of which over half were Fc receptor-positive.

‡Graft versus host (GvH) spleen refers to spleen cells from lethally irradiated (850 R) C₃D₂F₁ mice injected with 50×10^6 DBA/2 spleen cells beforehand to obtain activated T cells¹⁹.

§The P815 line as well as the others are long term established tissue culture cell lines. The P388D₁ cells¹⁰ were provided by Dr H. Koren, National Institutes of Health. The FVL (Friend virus leukaemia) cells were donated by Dr J. C. Kennedy, Queen's University, Department of Pathology. Cells were taken from both stationary and log-phase cultures which account for part of the variability in Fc rosette counts after NMS.

¶DBA/2 mice were injected with 10^6 SaD/2 tumour cells, the tumours removed 3–4 weeks later and a single cell suspension made as described previously¹².

||The SaD/2 tumour cell suspensions were treated with iron filings and magnetism as described previously¹² to remove macrophages. Mean of two experiments recorded.

some of which may be Fc receptor-positive⁹, behaved like their normal counterparts with regard to anti-H-2-mediated inhibition of Fc rosette formation (Table 1).

Thus, activated macrophages, in contrast to normal macrophages, normal lymphocytes and activated lymphocytes are capable of forming Fc rosettes after treatment with relevant anti-H-2 serum. These animal experiments can be mimicked using cells from long term established tissue culture lines which are Fc receptor positive. Whereas cells from the L1210 myeloid leukaemia, a Friend-virus-induced erythroleukaemia, and the P815 mastocytoma were all totally inhibited, the P388D₁ cell line—which is a macrophage-like cell line¹⁰—retained its full Fc rosetting ability (Table 1).

The possibility of exploiting the observation that activated macrophages can still form Fc rosettes after anti-H-2 treatment as a 'marker' to screen cell suspensions for their presence would seem verified by further experiments (Table 1) in which cell suspensions made from solid fibrosarcoma tumours—which are often known to contain many macrophages^{11,12} which can be in an activated state¹³—were tested for the presence of phagocytic cells and anti-H-2 resistant Fc rosette-forming cells. The respective proportions of these cells were very similar, and previous removal of phagocytic cells from the tumour cell suspensions left a residual population of Fc rosette-forming cells which were capable of being totally inhibited by anti-H-2 serum pretreatment.

The reasons why activated macrophages retain their Fc rosetting ability after anti-H-2 treatment are not clear. Some possibilities include: a possible significant drop in the relative density of H-2 antigens on macrophages when they become activated; the number and avidity of Fc receptors are both known to increase on macrophages when they become activated^{4,14}; an antigen, other than H-2, to which antibodies in the polyspecific anti-H-2 sera can react, may be present on normal macrophages but not on activated macrophages, thus effectively diluting the potency of the anti-H-2 sera. If this is the case, the Ia

antigens may be involved, as these are present on normal macrophages^{15,16} but may not be on activated macrophages¹⁷.

The first possibility may explain why activated macrophages seem relatively resistant to the cytotoxic effects of anti-H-2 serum and guinea pig complement¹² (GPC). As shown in Fig. 1, killing of such cells requires a much more potent source of complement (in this case rabbit serum¹⁸) for the cells to be killed. In contrast, normal lymphocytes are easily killed by anti-H-2 serum and GPC, as are activated lymphoid cells^{19,21}. These results indicate caution should be exercised when using anti-H-2 sera and GPC as cytotoxic markers on lymphoreticular cell populations as one may inadvertently select for any activated macrophages that may be present.

In summary, it has been found that activation of macrophages is accompanied by an increased resistance of these cells to anti-H-2 serum mediated cytotoxicity and inhibition of Fc (EA) rosette formation; the latter observation can apparently be applied successfully as a simple marker to detect activated macrophages.

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H-2-like specificities of foreign haplotypes appearing on a mouse sarcoma after vaccinia virus infection

ATTENTION has focused recently on the interaction of viruses and histocompatibility antigens, and it has been proposed that this relationship may be central to immune surveillance¹⁻³. We report here the new expression of H-2 specificities apparently of other haplotypes on the surface of a chemically induced mouse tumour cell line following vaccinia virus infection.

A sarcoma induced by 3-methylcholanthrene (Meth A, H-2^d) was passaged in syngeneic mice (BALB/c, H-2^b). At the time of passage, the recipients were also infected intraperitoneally with vaccinia virus. Control BALB/c mice received tumour but no virus. After 7 d tumour cells were taken from the peritoneal cavity from both infected and control mice, washed and tested for a wide range of H-2 antigenic specificities—both public and private. We used a new microradioassay that involves antibody-complement treatment of the target tumour cells followed by testing for the uptake of ¹⁴C-uridine in a postlabelling assay (manuscript in preparation).

We found the normal H-2^d haplotype specificities in the control tumour (private H-2D.4, H-2K.31 and public H-2.3, 8, 28, and 35), whereas in the tumour from infected animals we repeatedly and reproducibly found reactions with anti-H-2 sera of restricted specificity apparently detecting ten additional private and three public specificities—normally present only in mice of other H-2 haplotypes (Table 1). The extra-reactivities seemed to be restricted to anti-H-2 sera, since normal mouse sera from various strains, antisera against non-H-2 antigens (Thy 1.2; Ly 4.2) and, interestingly, antisera against I-region-associated (Ia) antigens did not react. The extra specificities were found 5-7 d after infection but after 2 weeks there was no difference between tumour cells from infected and control animals. This time course roughly parallels that of the

Table 1 Cytotoxic activity of anti H-2 sera against Meth A sarcoma cells grown in syngeneic mice in the absence (control) or presence of vaccinia virus

Pretreatment of tumour cells with Sera* (Haplotype) C'			Meth A control ¹⁴ C-uridine uptake c.p.m. ± s.d. % Reduction		Meth A passaged with vaccinia virus ¹⁴ C-uridine uptake c.p.m. ± s.d. % Reduction	
—		—	3.861 ± 181		3.364 ± 169	
—		+	3.641 ± 125		3.790 ± 160	
Private						
anti H-2D.2	(b,g,h,j)	+	3.800 ± 39	0	2.577 ± 361	32
anti H-2D.4†	(a,d,i,t,u,y)	+	176 ± 50	96	131 ± 28	97
anti H-2.9	(f)	+	3.685 ± 143	0	3.827 ± 215	0
anti H-2D.12	(s)	+	2.366 ± 88	35	607 ± 31	84
anti H-2K.15	(j)	+	1.383 ± 94	62	589 ± 81	85
anti H-2.16	(p)	+	1.379 ± 120	62	733 ± 245	81
anti H-2K.17	(q,y)	+	2.427 ± 81	34	1.041 ± 170	73
anti H-2.18	(r)	+	2.459 ± 134	32	3.030 ± 245	21
anti H-2.19	(s,t)	+	3.614 ± 451	0	833 ± 115	78
anti H-2K.20	(u)	+	3.699 ± 188	0	3.982 ± 417	0
anti H-2K.23	(a,h,k,m)	+	3.660 ± 81	0	1.326 ± 381	65
anti H-2D.30	(m,q,v)	+	3.735 ± 53	0	947 ± 120	75
anti H-2K.31†	(d,g,o)	+	423 ± 171	90	141 ± 14	96
anti H-2D.32	(k,o)	+	3.648 ± 280	0	683 ± 77	82
anti H-2K.33	(b,i)	+	4.035 ± 55	0	1.749 ± 251	54
Public						
anti H-2.1		+	3.604 ± 231	0	580 ± 62	88
anti H-2.3†		+	1.529 ± 183	58	120 ± 17	97
anti H-2.5		+	3.880 ± 371	0	1.630 ± 313	57
anti H-2.8†		+	315 ± 32	92	97 ± 32	98
anti H-2.11		+	2.912 ± 192	20	760 ± 92	80
anti H-2.13†		+	112 ± 37	98	121 ± 38	97
anti H-2.25		+	3.622 ± 431	0	3.947 ± 428	0
anti H-2.28†		+	132 ± 44	98	93 ± 17	98
anti H-2.35†		+	151 ± 30	98	108 ± 35	98
Others						
normal mouse serum		+	3.727 ± 91	0	3.401 ± 411	15
anti-Thy 1.2		+	2.953 ± 10	19	3.011 ± 110	20
anti-Ly 4.2		+	3.551 ± 184	0	3.677 ± 315	3
anti-Ia (A.TH anti A.TL)		+	3.042 ± 201	15	3.127 ± 414	27

5 × 10⁵ Meth A tumour cells were injected with or without vaccinia virus (10⁷ infectious units of Lister strain) into BALB/c mice. 7 d later the tumour cells from both groups were collected from the peritoneum and washed. 2 × 10⁴ macrophage-depleted Meth A tumour cells (infected or control) in 50 µl culture medium were incubated in round-bottom wells of sterile microtitre plates with 1 µl of antiserum for 1 h at 4 °C and then 30 min with 50 µl of prediluted (1/10) rabbit complement at room temperature (C'). 10 µl ¹⁴C-uridine (0.3 µCi ml⁻¹; 402 mCi mmol⁻¹) were added to the plates for a further 16 h. The cultures were collected in a semiautomatic machine. The results are expressed as the percentage reduction in uridine uptake in test samples compared with control samples with complement and no antibody. Similar results were obtained by measuring ¹⁴C-thymidine uptake. The results were also reproduced in a conventional microcytotoxicity titration using serially diluted antisera.

*The anti-H-2 sera represent sera of the D series raised under contract by the Transplantation Immunology Branch of the National Institutes of Health. For details see National Institutes of Health catalogue and supplements²³; anti H-2D.12 and anti-H-2.11 reacted weakly with normal Meth A tumour cells and not with H-2^d lymphocytes. Anti H-2K.15, H-2K.17 and H-2.18 seem to contain an unidentified antibody which reacted weakly with H-2^d lymphocytes. Anti-H-2.16 also contains anti 34 which could explain the cytotoxicity against Meth A control. A.TH anti-A.TL was obtained from Dr Chella David, the other sera were raised in the London Hospital Medical College.

†Private specificities present on H-2^d lymphocytes: H-2D.4, H-2K.31; public: H-2.3, 8, 13, 28, 35 (ref. 22).

Table 2 Absorption of cytotoxic anti-tumour activity from anti-H-2 sera by normal lymphocytes carrying the appropriate H-2 antigenic determinants

Antisera	Absorbing cells* from	Meth A control		% Reduction of ¹⁴ C-uridine† uptake by Meth A + vaccinia		Gardener	
		Predicted result	(H-2 ^d)	Predicted result	(H-2 ^d)	Predicted result	(H-2 ^k)
Anti-H-2D.4	—	+	96	+	95	—	2
Anti-H-2D.4	B10.BR (H-2 ^k ; D.4 neg.)	+	96	+	93	—	0
Anti-H-2D.4	B10.A (H-2 ^a ; D.4 pos.)	—	6	—	0	—	0
Anti-H-2D.32	—	—	0	+	82	+	89
Anti-H-2D.32	B10.A (H-2 ^a ; D.32 neg.)	—	0	+	75	+	87
Anti-H-2D.32	B10.BR (H-2 ^k ; D.32 pos.)	—	0	—	9	—	4
Anti-H-2.1	—	—	0	+	88	+	89
Anti-H-2.1	B10 (H-2 ^b ; 1 neg.)	—	0	+	87	+	91
Anti-H-2.1	B10.A (H-2 ^a ; 1 pos.)	—	0	—	18	—	14

*100 µl of 1/10 diluted antiserum was absorbed with 10⁸ spleen cells from B10, B10.BR or B10.A mice for 1 h at 4 °C and tested in presence of complement for cytotoxic activity against normal Meth A, Meth A from vaccinia-infected mice (day 7) and Gardener lymphoma. The tumour cells were then postlabelled by culturing them overnight with ¹⁴C-uridine.

†¹⁴C-uridine uptake in the controls (treated with complement alone) was for Meth A 3.114±251, for Meth A + vaccinia 2.296±132 and for Gardener lymphoma 2.316±613.

induction of cytotoxic T cells against vaccinia-infected syngeneic target cells⁴.

The results in Table 2 show that the extra-reactivity with anti-H-2D.32, directed against a private specificity of the H-2^k haplotype, could be absorbed with spleen cells from B10.BR (H-2^k, D32 positive) but not from B10.A (H-2^a, D.32 negative) mice. Furthermore, cytotoxic activity of anti-H-2.1 could be absorbed with cells from B10.A (positive for 1, a public specificity) but not from C57BL/10 (B10, negative for 1). But we also had experiments in which there was nonspecific removal of activity by normal lymphoid cells. It is unlikely that the extra-reactivities are due to anti-virus activity in the sera because anti-virus activity could not be removed by such a procedure^{3,6}.

In theory new antigenic expression can be due to: (1) direct alteration of the H-2 specificities on the cell surface as suggested by Doherty and Zinkernagel²; (2) introduction of new surface determinants coded for or induced by the virus genome; these determinants could cross react with and have similar properties as histocompatibility antigens^{7,8}; (3) derepression of pre-existing genetic information for H-2 specificities of different haplotypes (see discussion by Martin⁹ and Bodmer¹⁰).

We have no evidence for alteration of the normal H-2 specificities on the Meth A tumour cell since the cells from infected mice could absorb anti-H-2 antibody directed against the private specificities of the syngeneic strain (H-2D.4 and H-2K.31). The next possibility of direct viral action modifying the 'H-2 profile' or creating new 'H-2 like' determinants is an attractive one which we can neither prove nor disprove. An even more attractive possibility, for which we also have no proof, is derepression. Derepression could explain findings in the TL system in mice¹¹ and the Gm allotype system in man¹², indicating that allelic differences may be the products of closely linked genes and the genetic polymorphism is exerted on the level of the control of whichever of the genes may become expressed¹⁰. With regard to our findings, it could be anticipated that the polymorphism breaks down when the virus interferes with the metabolism of the tumour cell.

It is not known whether other H-2-associated polymorphisms, like for instance lymphocyte-activating determinants (LADs) are also affected in this manner but some findings point in this direction: (1) leukaemic cells were able to stimulate syngeneic remission lymphocytes^{13,14}; (2) during virus infection there was a transient stimulation in the mixed lymphocyte reaction between HLA identical lymphocytes (J. R. Salaman and H. Festenstein, unpublished); (3) influenza virus-infected lymphocytes stimulated syngeneic lymphocytes¹⁵; (4) the abnormally high stimulation of normal lymphocytes by human cell lines,

most likely infected with Epstein-Barr virus¹⁶. In this context particularly, it is interesting that we could not detect the emergence of Ia antigens on the infected tumour cells (Table 1).

Many questions are raised by these findings, particularly concerning the basis of surveillance against infections and tumours. The findings also highlight the role of the various components of the major histocompatibility system in alloimmune interactions¹⁷. If some tumours were to express one kind of H-2-like determinant rather than another, would this enable a tumour to grow better in some instances and less well in others, as recent reports^{8,18} suggest? We have preliminary data indicating a suppressive effect of vaccinia virus on tumour growth and there is a recent report on a similar effect of vaccinia virus on human melanomas¹⁹. Relevant also in this context are experiments showing syngeneic graft rejection after infection by lymphomagenic virus²⁰. Derepression of H-2 and possibly non-H-2 transplantation systems could be a normal surveillance mechanism analogous to the somewhat artificial situation of an alloimmune interaction. It also raises the question whether changes in immunogenicity induced chemically²¹ or by irradiation (paper presented by J. McBurney and T. R. Munro, Second European Immunology Meeting, Amsterdam, 1975) could have similar mechanisms.

Our observations may point to the possibility of inducing rejection in one situation (tumours) and to provoking loss of immunogenicity in others (transplants). If this can be done, we may approach an understanding of "the pre-occupation of the immune system with histocompatibility antigens"²¹ and the surveillance of transplants and tumours.

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Surface membrane alterations in somatic cell hybrids of neuroblastoma and glioma cells

SOMATIC cell hybrids have been used to discern many properties of parental cells. Genes for neuronal properties can be activated in hybrids formed by fusion of mouse neuroblastoma and rat glioma cells (refs 1 and 2, and B. Hamprecht, T. Amano and M. Nirenberg, personal communication). These cell hybrids resemble the parent neuroblastoma cells in many of their properties as they can be induced to extend long neurites and possess electrically excitable membranes¹. In addition the cell hybrids express other neuronal characteristics that are less pronounced in the parental cells, such as choline acetyltransferase¹, and morphine receptors². Many of the above properties suggest alterations in the surface membrane. We have shown previously that a particular group of glycopeptides is more prominently expressed on the cell surface in clones of mouse neuroblastoma which have the ability to undergo morphological differentiation than in clones that are unable to extend neurites³. In the present report, we show that this group of glycopeptides is abundant in the surface membranes of the hybrid cells.

The parent cell lines were: N18TG-2, a mouse neuroblastoma clonal line resistant to 6-thioguanine⁴ and C6BU-1, a 5-bromodeoxyuridine-resistant clone of rat glioma⁵.

Fig. 1 Chromatography on Sephadex G-50 of Pronase-digested trypsinates from mouse neuroblastoma clone N18TG-2 and hybrid NG108-15 cells grown in the presence of L-¹⁴C- or ³H-fucose, respectively. Trypsinates were combined, digested with Pronase and chromatographed as described³. Sephadex G-50 profile of Trypsinate A (loosely-associated glycopeptides) (a) and Trypsinate B (tightly-associated glycopeptides) (b) from N18TG-2 (○) and NG108-15 (●) cells. BD represents fractions in which Blue Dextran 2000 was eluted. Phenol red eluted at fraction 105.

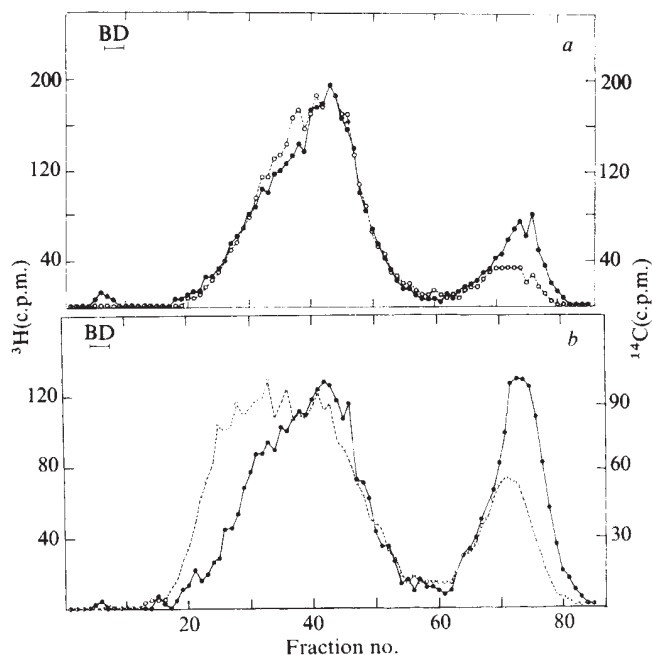
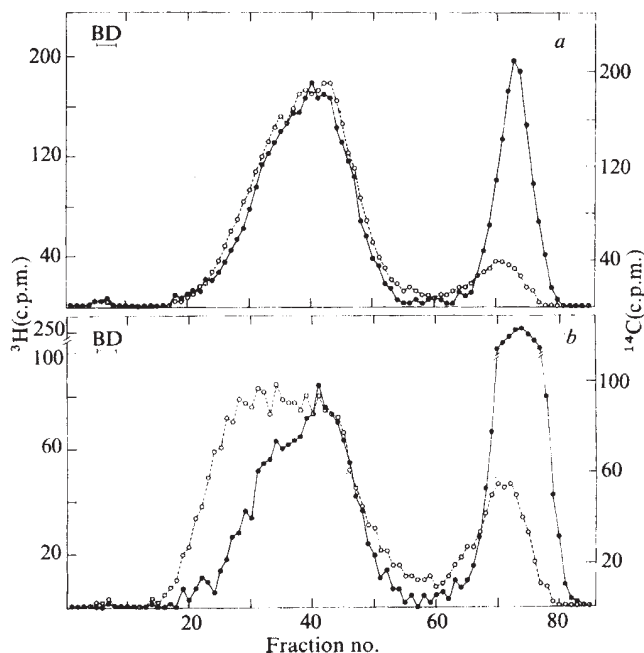


Fig. 2 Chromatography on Sephadex G-50 of Pronase-digested trypsinates from mouse neuroblastoma N18TG-2 and hybrid NG108-5 cells grown in the presence of L-¹⁴C- or ³H-fucose, respectively. Trypsinate A (a) and Trypsinate B (b) from N18TG-2 (○) and NG108-5 (●) cells. Details are described in Fig. 1.

Hybrids of these parental lines, NG108-5 and NG108-15 were obtained by Sendai virus-induced fusion of the neuroblastoma and glioma clones (B. Hamprecht, T. Amano and M. Nirenberg, personal communication). All these cells were obtained from Dr M. Nirenberg, NIH. The modal chromosome numbers for C6-B4-1 and N18TG-2 are 39 and 83 respectively, whereas the hybrids have chromosome numbers higher than the sum of the parental cells. These and other characteristics of the hybrids have been reported^{5,6}. The cells were grown to confluency in Dulbecco's modified Eagle's medium (DMEM) with 10% foetal calf serum, at which time the glycoproteins were made radioactive by incubating the cells for 2 d in medium containing L-³H- or ¹⁴C-fucose (4.8 Ci mmol⁻¹ and 57 mCi mmol⁻¹, respectively, Amersham). These procedures as well as those for collecting cells have been described³. The membrane glycopeptides were removed from the cell surface by sequential trypsinisation since different groups of glycopeptides are distinguished by this procedure³. One group is preferentially removed by the first trypsinisation step (Trypsinate A) suggesting that these glycopeptides are more accessible or loosely associated with the membrane⁷. Trypsinate B, which is removed with the second trypsin treatment, contains the more tightly-associated glycopeptides. All radioactive trypsinates to be compared were combined, digested exhaustively with Pronase (Calbiochem) and chromatographed on a column of Sephadex G-50 (ref. 3). Choline acetyltransferase (CAT) and acetylcholinesterase (AChE) were assayed according to Blume *et al.*⁸. Duplicate extracts at two concentrations of cells in the confluent state were used. The formation of acetylcholine by CAT was verified using electric eel AChE⁹. Specific AChE was determined by including 10⁻⁵ M BW-284C51 dibromide (Wellcome) in the assay mixture.

The chromatographic profiles of the loosely-associated glycopeptides (Trypsinate A) from the surface of the parent neuroblastoma and hybrid cells were found to be similar (Figs 1a and 2a), and slightly different from that of the parent glioma cells (Fig. 3a). On the other hand, the pattern of the more tightly-associated glycopeptides

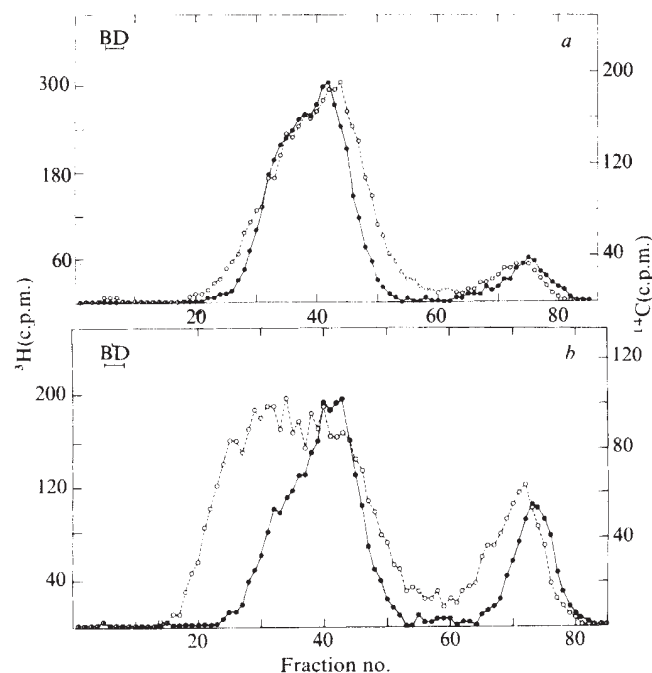
Table 1 L-³H-fucose incorporation into parental and hybrid cells

Clonal lines	L- ³ H-fucose incorporation		Trypsinate	
	c.p.m. per 10 ⁵ cells	c.p.m. per µg protein	A	B
Parents				
Mouse neuroblastoma N18TG-2	2,150	63	4	3
Rat glioma C6BU-1	235	15	8	4
Hybrids				
NG108-5	13,134	154	7	2
NG108-15	4,235	74	7	4

Confluent cultures, in 60-mm dishes, were incubated in 7 ml medium containing L-³H-fucose for an additional 51 h (477,500 c.p.m. per ml medium). Cells were washed four times with cold 0.16 M NaCl and removed from the monolayer with 1 ml 0.25% trypsin solution (3 min at room temperature). To the cell suspension, 1 ml medium containing 10% foetal calf serum was added and aliquots removed for radioactive counting. Values for the trypsinates were obtained from another set of radioactively labelled cultures. Sequential trypsinisation was carried out as described previously³.

(Trypsinate B) from the parent neuroblastoma cells differed markedly from those of the hybrid and glioma cells (Figs 1b, 2b and 3b). The chromatographic profiles of the tightly-associated glycopeptides from the parent neuroblastoma cells may be divided into early eluting groups (Fig. 1b, fractions 15–30), more slowly eluting groups (Fig. 1b, fractions 31–50) and dialysable material (fractions 65–80). The latter includes free fucose, and the significance of this material requires further investigation. The early eluting glycopeptides were missing from the material derived from the surface of the hybrid cells (Fig. 1b and 2b) and the parent glioma cells (Fig. 3b). In contrast, the more slowly eluting glycopeptides (fractions 31–50) were similar in all of the cell lines examined. This latter group (fractions 31–50) was also prominent in the neuroblastoma clones which are capable of morphological differentiation and less pronounced in the surface glycopeptides from axon-minus cells³ or these cells in logarithmic phase of growth (M.C.G., Y.K. and U.Z.L., unpublished).

Fig. 3 Chromatography on Sephadex G-50 of Pronase-digested trypsinates from mouse neuroblastoma N18TG-2 and rat glioma C6BU-1 cells grown in the presence of L-¹⁴C- or ³H-fucose, respectively. Trypsinate A (a) and Trypsinate B (b) from N18TG-2 (○) and C6BU-1 (●) cells. See Fig. 1.



In accordance with previous results (B. Hamprecht, T. Amano and M. Nirenberg, personal communication), high levels of CAT were found in the hybrid cell lines, whereas the activity of this enzyme in both parent cells was low (143,160, 5.8 and 3.8 pmol per mg protein per min for NG108-5, NG108-15, N18TG-2 and C6BU-1, respectively). The expression of this enzyme was also reported to be activated in a hybrid clone of mouse neuroblastoma NA and human fibroblasts¹⁰. In addition, AChE activity was expressed in the two hybrids and the parent neuroblastoma cells (14,700, 7,480 and 7,980 pmol per mg protein per min). The parent glioma cells had less activity (82 pmol per mg protein per min).

Less radioactive L-fucose was incorporated into the glioma cells than into either the hybrid or neuroblastoma cells, either expressed as per cell or per mg cell protein. The latter takes into account the size difference of the cell lines (Table 1). Since the percentages of the total incorporated fucose which were removed by the trypsinisations were similar, the fucose-containing glycoproteins may be deficient in the membranes of the glioma cells. The cells were grown in the presence of radioactive fucose for 51 h, so the amounts probably do not reflect increased turnover of the membrane glycoproteins. These results suggest that in this phenotypic expression of the fucose-containing glycoproteins the traits more characteristic of the neuroblastoma cells continue to be preserved or even activated in the hybrids. Closer scrutiny of the fucose-containing glycopeptides using chromatographic profiles (Figs 1b and 2b), however, showed only a partial preservation in the hybrids of the phenotypic expression of the neuroblastoma cells. More detailed analyses of the glycoproteins will be required to determine if the glioma phenotype is preserved at all.

The hybrid cell lines were shown to undergo biochemical, morphological and electrical differentiation (B. Hamprecht, T. Amano and M. Nirenberg, personal communication). For this reason, we anticipate that analysis of the membrane glycopeptides from a variety of hybrids will reveal which glycopeptides are associated with the acquisition of these properties. We conclude from the present studies that the glycopeptides which elute early from the Sephadex G-50 column may not be connected with the phenotypic expression of the differentiated state in neuroblastoma cells, since they are missing from the surface of the hybrid cells. It follows that the slowly eluting group of glycopeptides may be associated with the ability to neuroblastoma cells to differentiate. This group consists of several glycopeptides⁷. Since the glioma cells also show a corresponding group of slowly eluting glycopeptides it may be possible with further chemical studies to distinguish between those which the cells may have in common and those which correlate with differentiation. The fact that hybrid cells do not possess the early eluting glycopeptides substantiates our earlier finding³ that the slowly eluting glycopeptides are characteristic of the differentiated state of neuroblastoma cells.

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Tumour promotor induces plasminogen activator

INFECTION of chick embryo fibroblasts with Rous sarcoma virus (RSV) induces a cell-specified plasminogen activator¹. Induction occurs with transforming viruses but not with lytic viruses or with oncornaviruses which are not themselves transforming². Similarly, many mammalian cell lines and embryo cultures transformed with either viruses or chemical carcinogens may be high producers of plasminogen activator in contrast to their untransformed counterparts^{3–6}. A correlation has been demonstrated between production of plasminogen activator and various features of the transformed phenotype, such as cell locomotion, morphology and loss of anchorage-dependent growth^{4,7}. Several established cell lines which are not highly tumorigenic or transformed by the usual criteria are, however, active producers of plasminogen activator; there are examples also of transformed or tumorigenic cell lines which do not produce significant levels of plasminogen activator^{3,8–10}.

We have been interested in the regulation of the expression of plasminogen activator in the expectation that an understanding of the mechanism of its control and an ability to alter its levels with exogenous agents would provide insights into its physiological significance in the

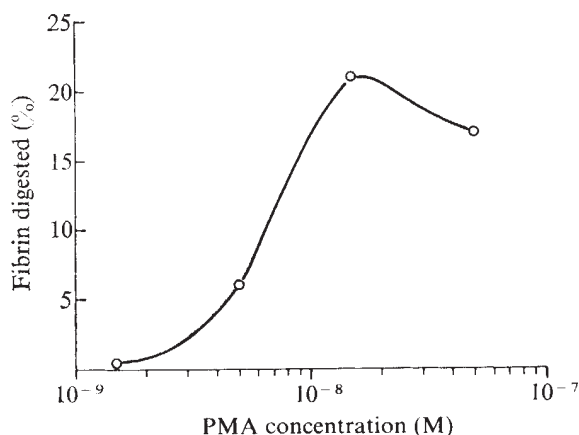


Fig. 1 A dose study of PMA in CEF cells. Subconfluent plates of CEF (approximately 5×10^6 cells per 9-cm dish) were re-fed growth medium containing PMA at the indicated concentrations. After 24 h cells were washed twice with phosphate-buffered saline and collected by scraping into 2 ml of swelling buffer (10 mM Tris, 10 mM NaCl, pH 8.0) without detergents. Lysates were diluted 1:10 into assay buffer (100 mM Tris, 10 mM NaCl, 3 mM NaN_3 , pH 8.0) and assayed in triplicate as described by Unkeless *et al.*¹ on fibrin-coated plates containing 40,000–70,000 c.p.m. of ^{125}I -fibrin ($10 \mu\text{g cm}^{-2}$) in the presence of 4 μg per ml human plasminogen previously purified by lysine-Sepharose affinity chromatography¹³. Aliquots were taken after 3 h and the solubilised fibrin determined by scintillation counting (Nuclear Chicago) in Aquasol (New England Nuclear Corp.) using the ^3H channel. Percentage fibrin digested was determined from the ratio of the counts released in the presence of sample (adjusted for fibrin released in the presence of plasminogen but in the absence of sample) to the total counts released by trypsin.

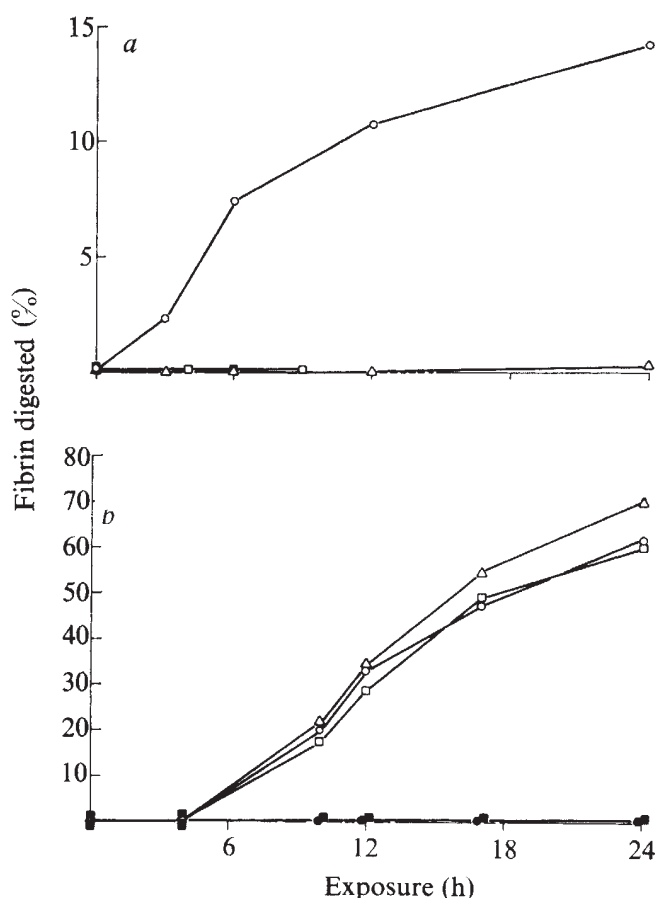


Fig. 2 Time course of induction. *a*, Cell lysate assay. Agents were added to replicate subconfluent plates of CEF at zero time and at the subsequent times indicated in the figure cells were collected and lysates assayed as described in Fig. 1. Δ , Untreated CEF; $\circ$, CEF treated with 5×10^{-8} M PMA; $\square$, CEF treated with 5×10^{-8} M PMA and $1 \mu\text{g ml}^{-1}$ actinomycin D. *b*, Intact cell assay. Assays were carried out according to Unkeless *et al.*². CEF (2×10^5) were plated in triplicate on to 6-cm plates coated with 50,000 c.p.m. ^{125}I -fibrin ($10 \mu\text{g cm}^{-2}$). The medium was Dulbecco's with 10% foetal calf serum. Six hours after plating (zero time), various agents were added to the plates. Aliquots were taken at the times indicated in the figure and percentage fibrin digested determined as described in the cell lysate experiments. $\bullet$, Untreated CEF; $\square$, CEF treated with 1.5×10^{-8} M PMA; $\circ$, CEF treated with 1.5×10^{-8} M PMA and 10^{-6} M phorbol; $\blacksquare$, CEF treated with 10^{-6} M phorbol; $\triangle$, CEF treated with 5×10^{-8} M PMA.

normal and malignant state. We reported that glucocorticoids inhibit expression of plasminogen activator in certain cell lines¹⁰. Troll *et al.* found that a phorbol ester, phorbol-12-myristate-13-acetate (PMA), a component of croton oil which causes epidermal inflammation, hyperplasia and tumour promotion in treated skin¹¹, also induces increased trypsin-like proteolytic activity in treated mouse skin¹². In view of these findings, we tested the effects of PMA on plasminogen activator levels in cultured cells, and report here that PMA is a potent inducer of plasminogen activator in chick embryo fibroblasts and HeLa cells.

Plasminogen activator was assayed by published methods^{1,2} (see legends to Figs and Table for details). The assay is based on quantification of the solubilisation of ^{125}I -labelled fibrin due to proteolytic digestion in the presence of plasminogen and sample. The sample may be either intact cells, plated directly on to a fibrin-coated dish, or a lysate of cells. In our studies, omission of plasminogen from the assay of PMA-induced cells or cell lysates reduced fibrinolysis by more than 95%, indicating that the fibrinolysis was plasminogen dependent. This justifies our referring throughout to the induction of a plasminogen activator.

Table 1 Maximum-fold increase in cell-associated plasminogen activator over endogenous levels after exposure to PMA

Cell	Origin	Time in culture	Maximum-fold increase in plasminogen activator
CEF	Chick embryo fibroblast	1-3 weeks	10
HeLa ²⁰	Human cervical carcinoma	20 yr	10
HEC	Hamster embryo cells	1-3 weeks	3
REC	Rat embryo cells	1-3 weeks	2
HTC ²¹	Rat hepatoma	10 yr	2
L ²⁰	Mouse fibroblast	20 yr	1

Exposure was for 24 h and ranged from 10 to 1,000 ng ml⁻¹. Cells were grown as monolayer cultures in either Dulbecco's modified Eagle's minimal essential medium (DMEM) with 5% foetal calf serum (FCS) (L cells), DMEM+10% FCS (CEF, HEC, REC, HeLa) or Ham's F12 Medium + 10% FCS (HTC). Embryo cultures were prepared by standard procedures^{22,23} from 9-12-d chick embryos, midterm pregnant rats or late term pregnant hamsters. Cell lysates were prepared and assayed as described in the legend to Fig. 1.

The dose-response curve of chick embryo fibroblasts (CEF) to PMA is shown in Fig. 1. Induction occurred at a concentration as low as 5×10^{-8} M PMA (3 ng ml⁻¹). Maximal induction was observed at 1.5×10^{-8} M. The time course of induction of plasminogen activator in CEF as assayed in cell lysates and intact cells is shown in Fig. 2a and b. Increased fibrinolytic activity in lysates (cell-associated plasminogen activator) was observed as early as 3 h after exposure to PMA (Fig. 2a). The longer delay in the appearance of extracellular fibrinolytic activity assayed with intact cells (Fig. 2b) reflects the lower sensitivity of this assay, since in Fig. 2b foetal calf serum was used as a source of plasminogen. When intact cells were assayed using foetal calf plasminogen, purified by lysine-Sepharose affinity chromatography¹³, rather than serum, induction was detected after 3 h (data not shown). Phorbol itself, which is inactive as a tumour-promoting agent¹¹, neither induced nor blocked induction (Fig. 2b). Actinomycin D completely blocked the response to PMA (Fig. 2a), suggesting that PMA induction requires RNA synthesis.

The induced levels of plasminogen activator gradually returned to control values within 12 h of removal of PMA. Curiously, the deinduction was also blocked by actinomycin D. In this regard, deinduction resembles the glucocorticoid-induced repression of plasminogen activator expression in rat hepatoma cells, which is also blocked by actinomycin D (ref. 10).

PMA is not toxic to early passage CEF at 5×10^{-8} M as judged by growth rate during a 4-d exposure; however, it did inhibit growth of late (eighth) passage CEF. A growth inhibitory effect of PMA on late passage CEF has also been reported by Diamond *et al.*¹⁴. In spite of the different effects on growth at early and late passage, the kinetics of induction of plasminogen activator were found to be the same at both passage times.

Several cell types were screened for response to PMA (Table 1). PMA produced approximately a tenfold elevation of plasminogen activator in HeLa and CEF. A two- to threefold increase was seen with hamster embryo cells, rat embryo cells and a rat hepatoma cell line, and no induction was found with the mouse L cell line. Unresponsive cell types may prove useful in an analysis of the mechanism of the PMA response.

Phorbol esters, in addition to causing epidermal hyperplasia, inflammation and tumour promotion, have various other biological effects. These include alterations in cell morphology, growth properties and increased ³H-choline incorporation in cultured cells¹⁴⁻¹⁶; mitogenesis in mouse 3T3 cells and lymphocytes¹⁷; a rapid but short lived increase in cyclic GMP in lymphocytes and mouse 3T3 cells¹⁷; decreased cyclic AMP levels in mouse skin¹⁸; and enhancement of cell transformation by chemical carcinogens in cell culture¹⁹. Our discovery that extremely low concentrations of PMA induce a marked increase in plasminogen activator in such diverse systems as CEF and

HeLa cells, together with the previously described *in vitro* effects of this compound, suggest that PMA induces a constellation of changes reminiscent of the phenotype of cells transformed by oncogenic viruses or chemical carcinogens, a major difference being that the effects of PMA are readily reversed when the agent is removed. An analysis of the mechanism of action of PMA in cell culture may, therefore, shed light on the general problem of malignant transformation.

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Evidence for inhibition of platelet activation in blood by a drug effect on erythrocytes

ADP released from erythrocytes causes adhesion and aggregation of platelets¹. This can presumably happen *in vivo*

when circulating red cells undergo haemolysis in pathological conditions^{2,3}, even though ADP is hydrolysed by enzymes present in blood⁴⁻⁷. More important pathologically, as a factor in thrombogenesis, is the possibility that ADP is also released from red cells which are structurally and metabolically normal. ADP might leak from circulating cells undergoing reversible deformations⁸ which are much increased by abnormal conditions of flow, for example in vessels narrowed by atherosclerosis.

Experimentally, the adhesiveness of platelets to glass is increased by the presence of erythrocytes without evidence of haemolysis; and the increase is abolished by apyrase which catalyses the dephosphorylation of ADP⁹. When heparinised blood from anaesthetised pigs is made to flow through extra-corporeal shunts, deposits of platelets form at branch points but nowhere else¹⁰; and no deposits form with platelet-rich plasma, indicating that haemodynamic effects on red cells suffice to increase platelet adhesiveness.

The resistance of erythrocytes to hypotonic haemolysis is increased by low concentrations of various drugs, including chlorpromazine and other phenothiazines¹¹. The mechanism by which these substances increase the resistance of red cells may alter their membrane so as to diminish or prevent the leakage of ADP; and if so, such an effect should show itself as a diminution of the reactivity of platelets in flowing blood. We have therefore determined the effect of chlorpromazine on the 'bleeding time' from a small hole in an artificial vessel, the extravasation being terminated, as *in vivo*, by a plug of platelets. The proposition that any diminution in platelet reactivity resulted from an effect of chlorpromazine on the red cells required that the bleeding time was prolonged by concentrations below those at which the drug inhibits the release reaction of platelets¹².

Human blood was obtained from the antecubital veins of apparently healthy volunteers who had not taken aspirin or any similar drug in the preceding week. The blood was mixed with trisodium citrate (final concentration 0.38%) as anti-coagulant. The concentration of platelets varied from 1.36×10^6 to 2.40×10^8 ml⁻¹. Blood was centrifuged at 280g for 15 min for preparing plasma containing 2.40×10^6 – 3.82×10^8 platelets ml⁻¹.

For each experiment, whole blood or platelet-rich plasma was pumped continuously from a reservoir through polyethylene tubing about 30 cm long and 0.3 mm internal diameter; the flow rate was 0.16 ml min⁻¹. The tube crossed a glass plate under a microscope (Leitz Laborlux) where it was continuously bathed in a stream of Tyrode's solution at 37.5 °C. A small cut was made through about one-third of the circumference of the tube and the duration of 'bleeding' into the bathing solution

was determined up to a maximum time of 600 s. Results are presented as median as well as mean ($\pm$ s.e.m.) bleeding times in Table 1.

For blood alone the median bleeding time was 130 s and for platelet-rich plasma 600 s. Thus, although the concentration of platelets was always higher in plasma than in blood, the bleeding time was much shorter with blood than with plasma; presumably the difference was due to some influences of the red cells.

Chlorpromazine (Hibernal; Leo AB Helsingborg, Sweden) was added to blood in the reservoir and the median bleeding time was more than doubled by the drug at 1.6×10^{-6} M, which is about 30 times less than the concentration required for halving the release reaction *in vitro*. Higher concentrations increased the bleeding time further, so that the 10-min limit was reached with about 1×10^{-5} M chlorpromazine.

β -Diethylaminoethyl-diphenylpropylacetate (Proadifen; SKF 525A, Smith, Kline and French Ltd, Welwyn Garden City, England) also increases the resistance of erythrocyte membranes¹³ and at 1×10^{-6} M increased the median bleeding time almost threefold.

The median bleeding time was also prolonged increasingly by the enzyme apyrase (Sigma) in concentrations of 0.5–50 μ g ml⁻¹; but less at 500 μ g ml⁻¹, possibly because of a nonspecific effect of so much added protein. The increase in bleeding time caused by apyrase is most simply accounted for by an accelerated breakdown of free ADP, preventing its activating effect on platelets.

The observations are compatible with our proposition that a drug such as chlorpromazine, which increases the resistance of red cells, can decrease the leakage of ADP from them in conditions in which this shows itself as a diminution in the activation of platelets in the blood. The hypothesis is thus that the activation of platelets by red cells depends on a chemical mechanism produced by released ADP. In flowing blood, red cells also affect platelets through a physical mechanism, by increasing the diffusivity of platelets by up to two orders of magnitude (see ref. 14). Presumably this greatly increases the frequency with which circulating platelets collide with vessel walls and such a physical effect is, indeed, required to account for the rate at which platelet thrombi are observed to grow when produced experimentally in living blood vessels¹⁵⁻¹⁷. The absence of platelet aggregation in plasma which is stirred at high rates¹⁸, however, indicates that the collisions on which the adherence of platelets to vessel wall and to each other depend do not by themselves make the platelets adhesive. That depends on their chemical activation by ADP.

Table 1 Effect of chlorpromazine, SKF 525A or apyrase on the median and mean bleeding times from a small cut in polythene tubes (for method see text)

	Addition	Concentration	Number of determinations	Bleeding time (s)			Bleeding time greater than 10 min (%)
				Median	Mean ($\pm$ s.e.m.)		
Blood	None (Controls)		70	130	208	19.7	7
	Chlorpromazine	(M)					
		1.6×10^{-6}	15	280	271	40.3	7
		3.2×10^{-6}	15	390	396	53.3	40
		8.0×10^{-6}	15	560	437	47.3	40
		1.6×10^{-5}	15	600	(509)	(41.4)	73
	SKF 525A	1.0×10^{-6}	10	300	340	64.4	10
		1.0×10^{-5}	10	540	400	70.4	40
		1.0×10^{-4}	11	600	(551)	(31.6)	82
	Apyrase	($\mu\text{g ml}^{-1}$)					
		0.5	10	360	349	74.3	40
		5.0	15	430	390	48.3	33
		50	14	600	(546)	(29.8)	57
		500	15	170	277	58.6	27
Platelet-rich plasma	None		28	600			96

Other possible explanations of our observations have to be considered. In altering the deformability of erythrocytes¹⁹, chlorpromazine might affect their flow properties such as to diminish the probability of platelet collisions. Any such effect is unlikely to make a measurable difference in our experiments; nor does it overcome the requirement that the platelets have to be activated before aggregating as an haemostatic plug. Another possibility is that chlorpromazine interferes with the aggregation of platelets after their activation by ADP, for example by competing with calcium²⁰ which is essential for aggregation. If this were the explanation, however, primary aggregation by added ADP should be inhibited by chlorpromazine in platelet-rich plasma, and this is not so¹². Furthermore, the concentrations of chlorpromazine which prolong the bleeding time are much lower than the calcium concentration required for platelet aggregation, even in the presence of citrate as anticoagulant.

Direct flow-mechanical activation of platelets has been demonstrated experimentally but in very different conditions²¹. Haemodynamic disturbances produce platelet thrombi in the blood channels of artificial organs such as kidneys or oxygenators, whereby their function is often terminated prematurely²². The conclusion that the red cells are primarily responsible for these thrombi accords with the fact that thrombus formation is similar in channels made of various materials as long as the geometry is the same. Our results, therefore, suggest a new way of prolonging the function of such artificial organs through the addition of an agent capable of inhibiting the release of ADP (and ATP) from erythrocytes. Such an agent should, of course, have as few other effects as possible, so that chlorpromazine, although effective, would not be the choice.

Our observations similarly suggest a new approach to the prophylaxis of those thrombotic diseases in which thrombus formation depends on platelets, as in a proportion of acute coronary thromboses. This approach would require the demonstration that the incidence of such a disease is diminished by drugs which, in clinically acceptable blood concentrations, do not inhibit platelet function directly and which inhibit the release of ADP experimentally from non-haemolysing erythrocytes during rheological stress. Our hypothesis may also explain the effect of dipyridamole and sulphinpyrazone in preventing increased utilisation of circulating platelets in potentially thrombogenic conditions²³. This cannot easily be accounted for by any direct action on platelets by either drug at its clinically effective concentration; instead, these drugs may act on the red cells to diminish their activation of platelets.

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Inhibition of plasmin-mediated fibrinolysis by vitamin E

BIOLOGICAL effects attributed to vitamin E include the maintenance of cell membrane integrity, inhibition of enzyme-dependent lipid peroxidation, participation in oxidative phosphorylation, and a general, non-enzymatic antioxidant effect¹. In spite of these observations, the physiological function of the vitamin remains obscure. Natural deficiency in man has been invoked in the pathogenesis of several disorders, with convincing evidence limited to a specific type of haemolytic anaemia². Therapeutically, vitamin E has its advocates in treatment of peripheral vascular and thromboembolic disease, although documentation for its efficacy is meagre³. Although it is considered non-toxic⁴, little is known about adverse effects of excessive dietary vitamin E.

Plasminogen is the plasma proenzyme which, on conversion to its active form, plasmin, is considered responsible for lysis of fibrin deposits resulting from physiological or pathological activation of the coagulation cascade⁵. In spite of interest in vitamin E as therapy for vascular disease, and the importance of coagulation and fibrinolysis in intravascular homeostasis⁶, information on its effects on these processes is limited. An anti-thrombin effect has been claimed⁶, but not confirmed³. Prolongation of plasma clotting time has been reported with vitamin E therapy, together with decrease in the expected increment in blood fibrinolytic activity which follows venous occlusion⁷. We report a direct effect of vitamin E on the fibrinolytic system, namely, *in vitro* inhibition of plasmin-mediated fibrinolysis at physiological concentrations of the vitamin and enzyme.

Fibrinolysis was assayed by a sensitive solid phase ¹²⁵I-fibrin tube method. This technique, described in detail elsewhere⁸, is based on release of radioactive degradation products from ¹²⁵I-fibrin (human), originally absorbed to polystyrene tubes as fibrinogen, and converted to non-cross linked fibrin by thrombin. Plasminogen was isolated from normal human plasma by affinity chromatography on lysine-Sepharose⁹, and subsequent stepwise⁸ or gradient elution¹⁰ with ε-aminocaproic acid (EACA). Plasmin was

Table 1 Effect of vitamin E on lysis of ¹²⁵I-fibrin by plasmin and other proteolytic enzymes

	¹²⁵ I-fibrin lysed (c.p.m.)		Δ
	- Vitamin E	+ Vitamin E	
Plasmin	9,968	1,634	-84%
Collagenase (Clostridiopeptidase A, Worthington)	18,941	17,870	-6%
Chymotrypsin (α-chymotrypsin, bovine pancreas, Worthington)	36,070	39,539	+10%
Trypsin (bovine pancreas, Worthington)	38,380	44,363	+16%
Pronase (bacterial protease, type VI, Sigma)	51,001	67,864	+33%

Enzyme (5 μg ml⁻¹) and vitamin E (1.7 × 10⁻⁵ M) were incubated in ¹²⁵I-fibrin coated assay tubes for 30 min at 37 °C (final volume, 0.2 ml in Tris-NaCl buffer). Results are means of duplicate assays. Assay tubes contained 145,000 c.p.m. of ¹²⁵I-fibrin (90,000 c.p.m. per μg fibrin).

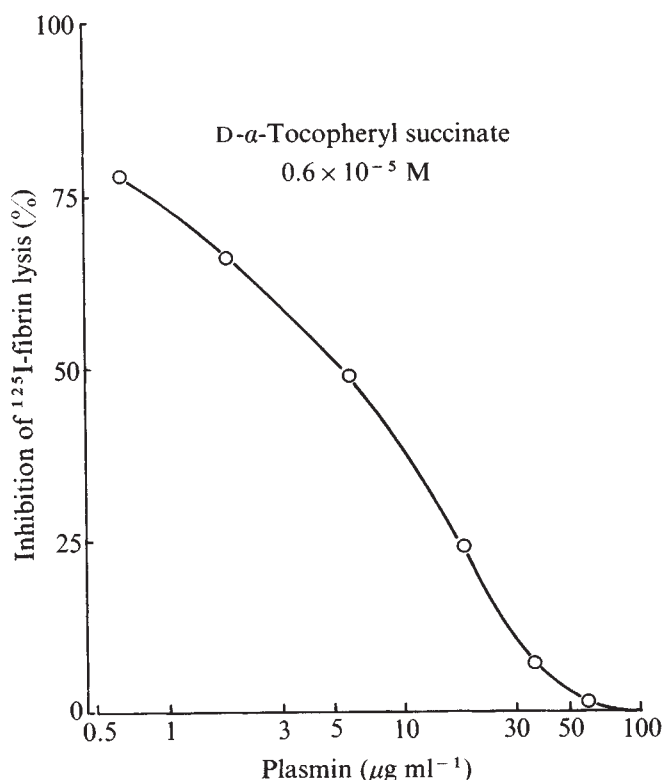


Fig. 1 Effect of vitamin E on lysis of ¹²⁵I-fibrin by human plasmin. Plasmin, at varying concentrations, was incubated with or without vitamin E (0.6×10^{-5} M) at 37°C for 30 min (final volume, 0.2 ml) in assay tubes coated with ¹²⁵I-fibrin (140,000 c.p.m. per tube, 90,000 c.p.m. per μg fibrin). Lysis in presence of vitamin E is expressed as percentage of lysis by plasmin alone. Nonspecific release of radioactivity with buffer alone (289 c.p.m.) was subtracted from experimental values. Release with vitamin E alone (2.5×10^{-5} M), 270 c.p.m. Experimental values (means of duplicate assays) at representative plasmin concentrations (0.6, 6 and 60 μg ml⁻¹, values with vitamin E in parentheses): 364 (84), 6,504 (3,205) and 12,684 (11,870) c.p.m., respectively.

in the form of spontaneously activated plasminogen⁸, as judged by absence of generation of additional activity by appropriate streptokinase or urokinase treatment. Plasminogen and plasmin were quantitated by absorbancy at 280 nm (ref. 11). Vitamin E, in the form of D-α-tocopheryl succinate (type VI, Sigma) was suspended in Tris-NaCl buffer (0.015 M Tris, 0.15 M NaCl, pH 7.4) at a concentration of 1 mg ml⁻¹, stirred for 15 min at room temperature, and filtered through Whatman No. 1 paper. Vitamin E content of the filtrate was quantitated by absorbancy at 292 nm (ref. 12), and colorimetrically by the tocopherol red reaction¹³, after preliminary alkaline saponification to yield the free phenol. Concentrations of vitamin E obtained in this way were 3×10^{-5} to 4×10^{-5} M.

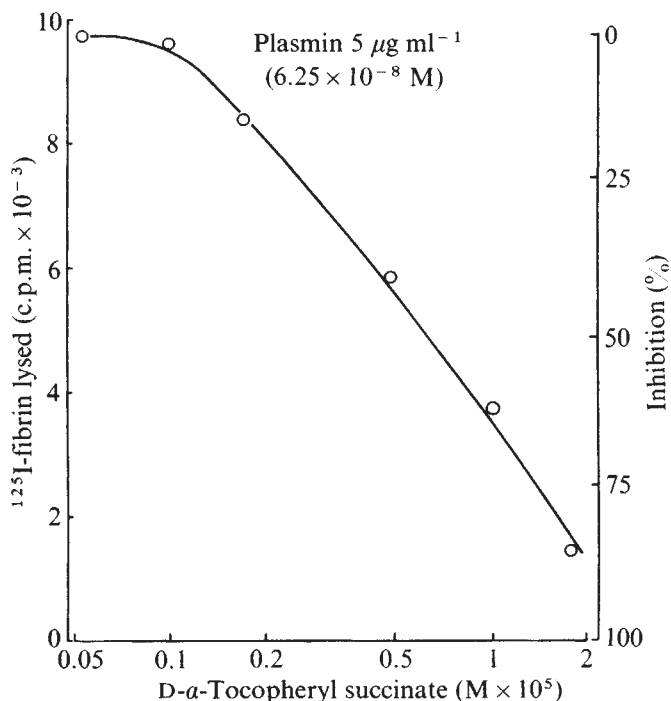
Vitamin E (0.6×10^{-5} M) produced 50% inhibition of fibrinolysis at a plasmin concentration of $5 \mu\text{g ml}^{-1}$, or 0.6×10^{-7} M (molecular weight of plasmin, 81,000 (ref. 14), an inhibitor-enzyme molar ratio of 100:1 (Fig. 1). Examination of the effects of varying concentrations of vitamin E (plasmin, $5 \mu\text{g ml}^{-1}$) was confirmatory (Fig. 2), with 50% inhibition at a vitamin E concentration of 0.5×10^{-5} to 0.6×10^{-5} M. Sodium succinate (5×10^{-3} M) or filtered buffer had no effect. Since vitamin E functions as an antioxidant¹, L-cysteine hydrochloride was tested. No effect on fibrinolysis was observed at concentrations from 10^{-7} to 10^{-3} M. Pretreatment of assay tubes with vitamin E (2×10^{-5} M, 37°C , 30 min) had no effect on subsequent ¹²⁵I-fibrin lysis. The effect of vitamin E on fibrinolysis by plasmin generated by streptokinase or urokinase activation of purified plasminogen⁹ was identical to that with spontaneously activated plasmin. The inhibition was also

demonstrable by the fibrin plate method¹⁷, in which non-radioactive fibrin is used as substrate (Fig. 3). Finally, addition of vitamin E to urokinase-stimulated normal plasma, at concentrations producing 50% inhibition of purified plasmin, resulted in 25% inhibition of ¹²⁵I-fibrin lysis.

Some observed effects of vitamin E esters, particularly the phosphate, which inhibits trypsin and papain¹⁵, have been attributed to their anionic nature rather than to tocopheryl group³, and may be mimicked (in the case of inhibition of hyaluronidase) by heparin and sodium dodecyl sulphate¹⁶. Neither of these agents, tested at 10^{-5} M, inhibited lysis of ¹²⁵I-fibrin by plasmin. Vitamin E did not inhibit lysis by four other proteolytic enzymes known to digest this substrate^{5,8} (Table 1), at similar molar concentrations, 0.5×10^{-7} M for collagenase to 3.3×10^{-7} M for trypsin). Additional evidence for the absence of an anionic effect has been provided by affinity chromatography experiments. D-α-tocopheryl succinate was coupled to aminohexyl Sepharose 4B (AH Sepharose, Pharmacia) by the carbo-diimide reaction. With a column (1 ml bed volume) of this conjugate, in which the succinyl carboxyl group is blocked, application of purified plasmin (260 μg) resulted in binding of 80%, and subsequent elution of 60% of the bound plasmin by buffer containing D-α-tocopheryl succinate. Finally, inhibition of fibrinolysis by plasmin does not appear to be due to the ester form of the vitamin, since similar inhibition was produced by the free phenol. Pure D-α-tocopherol (Eastman Kodak), dissolved in ethanol because of its insolubility, was added to and incubated with plasmin ($5 \mu\text{g ml}^{-1}$, 30 min at 37°C). Fifty per cent inhibition of plasmin lysis of ¹²⁵I-fibrin occurred at a free phenol concentration of 1.6×10^{-5} M, which compares with similar inhibition by the succinate ester at 0.6×10^{-5} M (Fig. 2). Lysis by plasmin plus ethanol (1.5% by volume) was 3,641 c.p.m., whereas lysis by plasmin in presence of D-α-tocopherol and ethanol was 1,855 c.p.m.

Reported levels of vitamin E in normal human plasma range from 6.5 to 15 μg ml⁻¹ (1.6 to 3.3×10^{-5} M)¹⁸, concentrations greater than those producing 50% inhibition of

Fig. 2 Effects of varying concentrations of vitamin E on lysis of ¹²⁵I-fibrin by plasmin ($5 \mu\text{g ml}^{-1}$). Assay conditions identical to those described in the legend to Fig. 1.



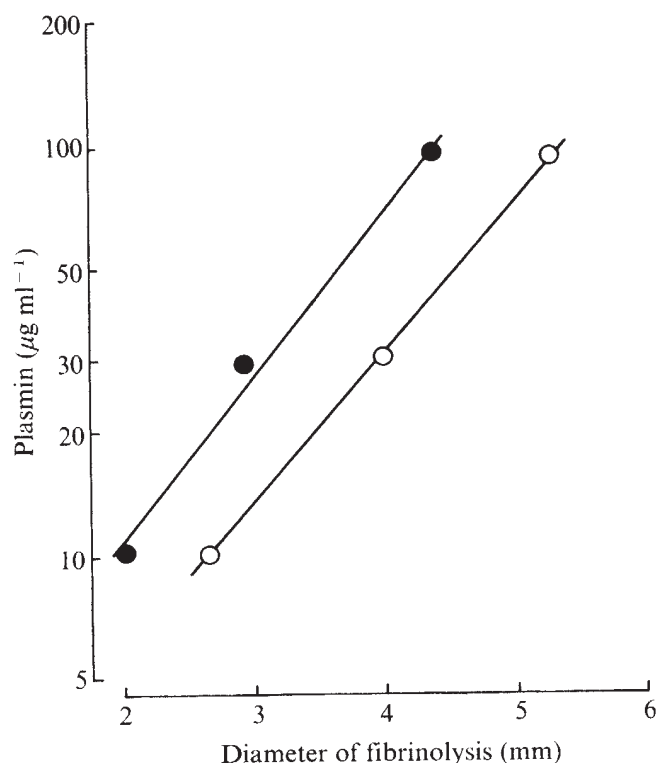


Fig. 3 Effect of vitamin E on lysis of fibrin by plasmin. Plasmin (10, 30 and 100 $\mu\text{g ml}^{-1}$), with or without vitamin E (1.5×10^{-8} M) was introduced into wells (5 μl per well) of heat-treated fibrin plates (Enzo-diffusion plates, Hyland). Diameter of lysis was measured after incubation at 37 °C for 6 h. Semilogarithmic plot plasmin concentration against diameter of lysis, with (●) and without (○) vitamin E. Operational zero point for the assay (no detectable lysis) is 2 mm, the diameter of the wells.

plasmin *in vitro* (Figs 1 and 2). Mean plasma levels of plasminogen-plasmin, determined immunochemically, are approximately 200 $\mu\text{g ml}^{-1}$ (ref. 19). Although comprehensive data on relative proportions of proenzyme and active enzyme in normal plasma are lacking, it is generally accepted that almost all is in the proenzyme form²⁰. In the present study, for example, plasminogen-plasmin, freshly prepared from normal plasma⁸, released 360 c.p.m. from ¹²⁵I-fibrin, representing less than 2% of the activity (18,911 c.p.m. released) of the same preparation (enzyme concentration, 5 $\mu\text{g ml}^{-1}$) after maximal urokinase activation. This estimated plasmin concentration (2% of 200 $\mu\text{g ml}^{-1}$, or 4 $\mu\text{g ml}^{-1}$) is of the same order as the concentrations examined here, and agrees with the observations of others²¹. Relative to other inhibitors, vitamin E produces 50% inhibition at the same concentration (approximately 10^{-5} M) as EACA or tranexamic acid (*trans*-4-aminomethylcyclohexanecarboxylic acid)⁸.

These observations may be pertinent to certain physiological and pathological phenomena, and to vitamin E therapy. Plasmin is normally inhibited by anti-plasmins in plasma. These include such macromolecules as α_2 -macroglobulin, α_1 -antitrypsin and C1 esterase inhibitor²². The inhibition of plasmin at physiological concentrations of vitamin E and enzyme raises the possibility that the vitamin may play a role in normal modulation of fibrinolysis. In addition, considering the broad substrate specificity of plasmin for many tissue proteins in addition to fibrin⁸, this effect may be relevant to foetal resorption in vitamin E deficient animals (that is, unopposed proteolytic activity), which led to the initial recognition of this vitamin²³. The *in vitro* inhibition of plasmin provides an explanation for the reported blunting, on oral vitamin E therapy, of the physiological increase in fibrinolytic activity after venous

occlusion⁷, a response considered to reflect increased conversion of plasminogen to plasmin, mediated by tissue activators released, presumably from vascular endothelium, in response to hypoxia²⁰. Further, fibrinolysis has been implicated in the development and spread of tumours²⁴. Both EACA and vitamin E inhibit fibrinolysis. Administration of EACA decreases metastatic spread of transplantable tumours in rabbits and rats²⁴, and there is a decreased incidence of carcinogen-induced lung tumours in vitamin E deficient mice when compared with normal mice, or deficient mice receiving vitamin E replacement²⁵. Finally, in view of the widespread use of the vitamin and its succinate and acetate esters, and the observed effects of oral administration on fibrinolysis *in vivo*⁷, the observations presented here may have implications for the consequences of dietary supplementation with the vitamin.

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Confusion between specific and nonspecific binding of carcinoembryonic antigen and blood-group antigens by eluted antibody preparations

Cross reactivity between carcinoembryonic antigen (CEA) and blood-group antigens is inferred from the observation that many human sera can bind labelled CEA. This is more common with sera of blood group O or B (that is containing anti-A antibodies), and binding may sometimes be inhibited by absorption of the sera with group A erythrocytes^{1,2}.

Following up these observations, Holburn *et al.*³ showed that hyperimmune human antisera against A, B, Le^a and Le^b antigens could bind 30–75% of added labelled CEA. They concluded that the extent of binding suggested that both CEA and blood-group antigens were present on the same molecule. Freedman, commenting on these experiments, pointed out that a hyperimmune antiserum will contain many other "sticky" substances as well as antibodies, and these might account for the results (paper read at meeting of British Society for Immunology, autumn,

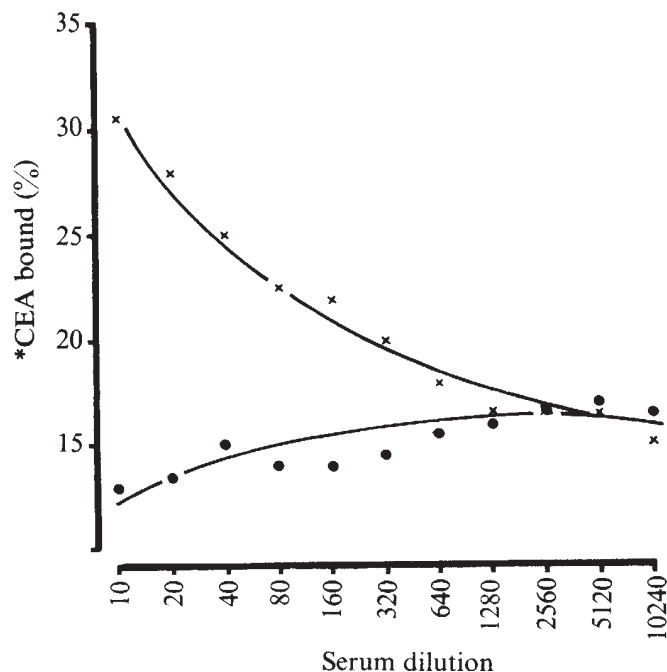


Fig. 1 Binding of untreated anti-A (x) and anti-B (●) to labelled *CEA, using Farr technique for separation.

1973). To exclude any extraneous binding substances his group in Montreal used "natural" anti-A iso-antibodies, which were adsorbed on to group A erythrocytes and eluted therefrom before use. Such antibody preparations could bind at least 45% of added labelled CEA as judged by the Farr technique¹ and at least 11% as judged by a solid-phase technique⁴. We have now found that rather than removing nonspecific binding capacity from antisera, the process of adsorption to red cells and elution may increase it.

A preparation of CEA, given by Professor A. M. Neville, Chester Beatty Institute, was labelled with ¹²⁵I by the chloramine T method⁵. Human anti-A and anti-B sera were obtained from Ortho Diagnostics (lots Pearl 02N0143 and Sally 11M1219, respectively). Samples of 60 ml of each were decomplexed at 56 °C for 30 min and added to 10 ml of washed packed fresh human erythrocytes; anti-A with A cells, anti-B with B cells. After overnight incubation at 4 °C, with gentle mixing, the massively agglutinated cells were washed three times with large volumes of cold 0.9% saline. They were then mixed with an equal volume of 20% bovine serum albumin in saline and gently agitated in a water bath at 56 °C for 10 min to elute the bound antibodies. The supernates were tested by haemagglutination to confirm the presence and specificity of the antibodies.

Each solution was then divided into three equal portions; one to be absorbed overnight at 4 °C with 10 ml washed packed A erythrocytes, one for similar absorption with group B erythrocytes and one to remain unabsorbed. The cells used for adsorption-elution and for absorption were taken from the same units of blood. Six solutions thus resulted: (1) eluted anti-A antibodies unabsorbed; (2) eluted anti-A antibodies absorbed with group A cells; (3) eluted anti-A antibodies absorbed with group B cells; (4) eluted anti-B antibodies unabsorbed; (5) eluted anti-B antibodies absorbed with group A cells; (6) eluted anti-B antibodies absorbed with group B cells.

The original anti-A and anti-B sera were used, without treatment, as further controls. Haemagglutination tests for antibody in all solutions demonstrated the presence and absence of the expected specificities.

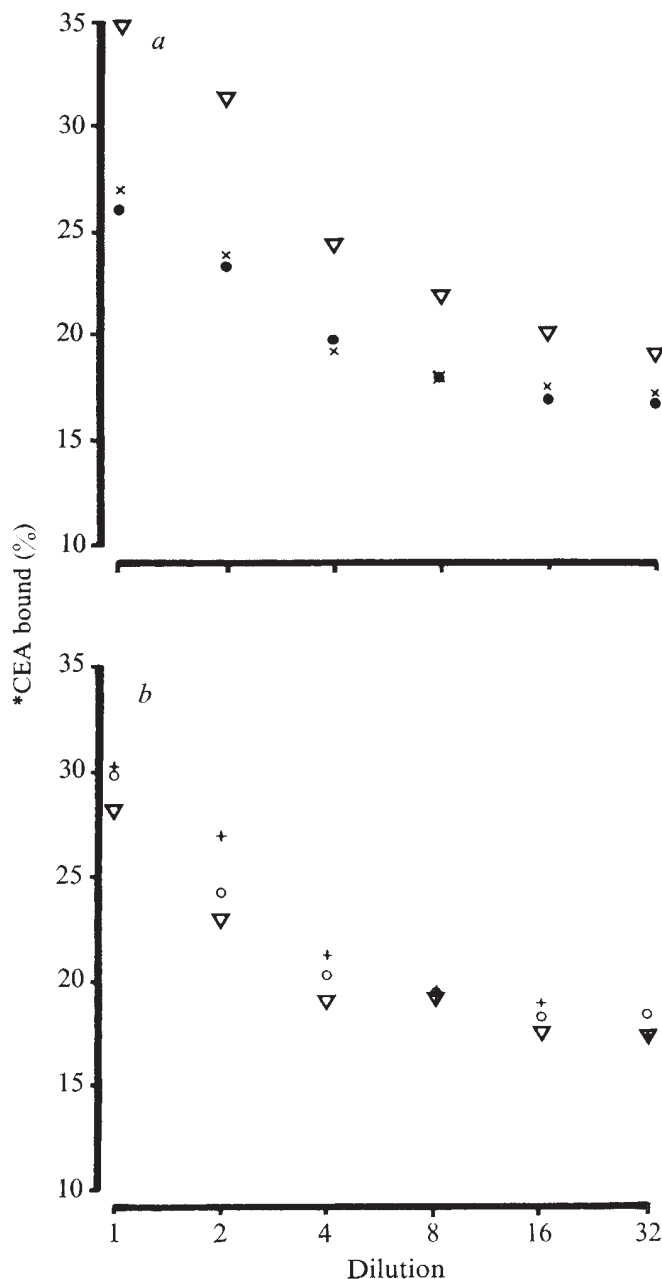
All sera were titrated against labelled CEA, the separation

system being half-saturation with ammonium sulphate in the presence of 0.5 ml of 0.1% bovine gamma globulin. The experiment was repeated on three occasions with the same results.

Figures 1 and 2 show that fresh untreated anti-A can bind at least 30% of the CEA (16% of the binding is nonspecific) while untreated anti-B has no significant activity—if anything, binding is slightly inhibited at a high concentration of serum. After adsorption and elution, all antibody solutions bind CEA to the same extent, irrespective of any subsequent absorption.

It might be argued that the Farr technique (half-saturation with ammonium sulphate) is an inappropriate separation method for this experiment, since a constant 10–20% of CEA itself is precipitated, without any anti-serum, in the presence of a 'carrier' protein (in this case,

Fig. 2 Binding of adsorbed-eluted anti-A and anti-B to labelled CEA, using Farr technique for separation. a: ▽, Anti-A absorbed with A cells; x, anti-A absorbed with B cells; ●, anti-A unabsorbed. b: +, anti-B unabsorbed; ○, anti-B absorbed with A cells; ▽, anti-B absorbed with B cells.



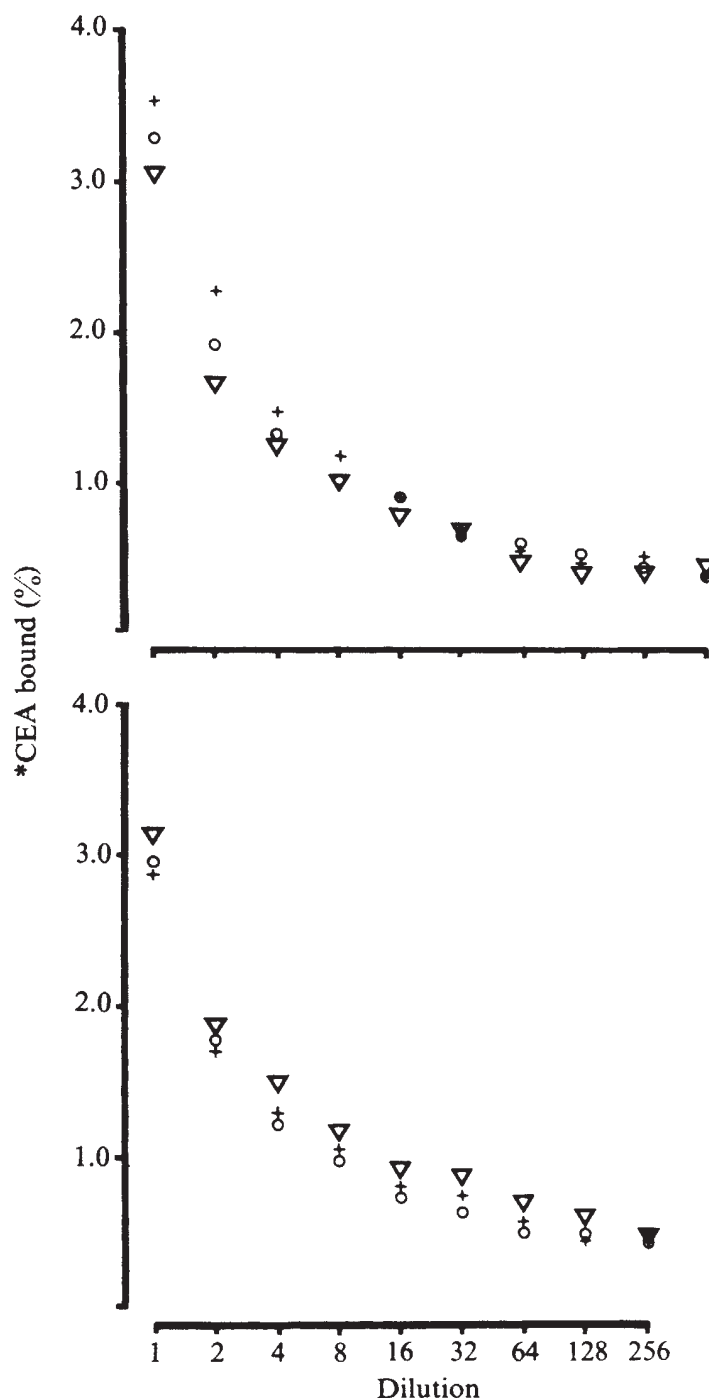


Fig. 3 Binding of adsorbed-eluted anti-A and anti-B to labelled CEA, using insoluble antibody preparations. a: +, Anti-A unabsorbed; O, anti-A absorbed with B cells; ▽, anti-A absorbed with A cells. b: +, anti-B absorbed with B cells; O, anti-B absorbed with A cells; ▽, anti-B unabsorbed.

bovine gamma globulin). Separation is therefore not clean and changes in binding capacity of the sera might be hidden. To answer this criticism, the six eluted antibody solutions were precipitated by 18% sodium sulphate and the globulins linked to microcrystalline cellulose (Sigma-Cell type 20) using the cyanogen bromide method⁶. The immunosorbents thus made were titrated against labelled CEA. Figure 3 shows that again there is no essential difference between absorbed and unabsorbed eluates.

These results show that adsorption and elution of anti-A

from group A red cells makes no difference to its ability to bind CEA, thus confirming the results of Gold *et al.*^{1,4}. On the other hand, absorption of this anti-A-containing eluate with group A red cells fails to reduce the binding capacity although its anti-A content becomes undetectable. This indicates that the CEA binding capacity of the eluate is not due to its anti-A activity. This conclusion is further supported by the development of CEA-binding capacity, previously absent, when anti-B is eluted from B cells. Again, this binding is not influenced by further absorption with either A or B cells.

The most likely interpretation is that human erythrocytes contain a 'sticky' non-antibody substance, extractable by heat elution, not removable by absorption with erythrocytes, which can bind to CEA. This may be the explanation for the finding⁷ that plasma which had been stored in contact with its cells had a higher apparent CEA level than the same specimen when fresh, and the subsequent finding⁸ that a CEA-like substance could be extracted from erythrocyte stromata. It must be remembered that the double antibody technique which was used would register a CEA-binding substance as if it were CEA.

The results reported here also throw doubt on the existence of cross reactivity between CEA and blood-group substances, since they discredit the use of erythrocyte-eluted antibodies for binding studies. Several reports favour such a negative view: (1) a monospecific anti-CEA serum does not agglutinate group A erythrocytes, and blood-group substances A and B do not inhibit CEA-anti CEA binding¹; (2) *N*-acetyl-D-galactosamine, the immunodominant sugar of A antigen, is conspicuously absent from most CEA preparations⁴; (3) treating CEA with anti-A and anti-i immunosorbents causes no reduction in its CEA activity⁹; and (4) although the blood-group precursors I and i can be found in extracts of colonic cancers, they can be separated from CEA by gel filtration¹⁰.

An alternative explanation is suggested by the association of CEA with mucus and the mucous coats of cells, both neoplastic and normal (to be published). One of the most striking physical properties of mucus is its cohesiveness. A CEA-binding substance is exuded by colonic cancer cells in tissue culture², and such cells are also known to exude CEA into the medium (as judged by techniques which do not confuse CEA-binding substances with CEA¹¹). Erythrocytes possess a mucous coat which they shed into the plasma as they become aged¹². We would suggest that both CEA and CEA-binding substance reside in mucus and in the mucous coats of cells, though not necessarily on the same molecule.

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Active proton transport stimulated by $\text{CO}_2/\text{HCO}_3^-$, blocked by cyanide

EXPOSURE of a cell to increased CO_2 levels causes a drop in the intracellular pH (pH_i) (ref. 1). The time development of this acidification has been monitored in snail giant neurones² and squid giant axons³ by means of glass microelectrodes. It was found that pH_i does not simply fall to a new steady value during CO_2 exposure, but, given sufficient time, will slowly recover; and that, on return to a CO_2 -free solution, a higher pH_i value (more alkaline) than the initial one is attained. The slow, secondary alkalisation observed during exposure to CO_2 cannot be accounted for by any passive mechanism, since the electrochemical gradients for H^+ , OH^- and HCO_3^- all favour further acidification. For example, during the alkalisation, the external pH (pH_o) in squid axons is typically 7.7 and pH_i 7.0; hence, the Nernst potentials for H^+ (E_H), OH^- , and HCO_3^- are all ~ -40 mV, compared with a membrane potential (V_m) of -60 mV. Thus, a proton extruding pump, or its equivalent, must be postulated. We now report that the rate of alkalisation is at least partially dependent on the CO_2 and/or HCO_3^- content of the bathing medium, that it is

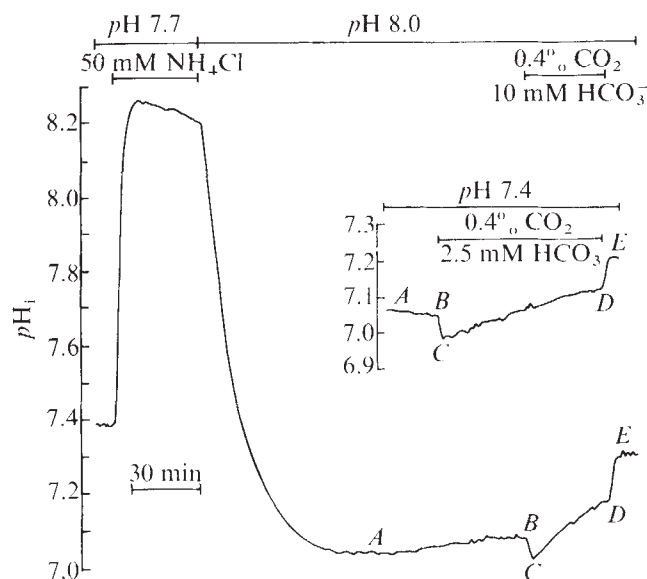


Fig. 1 Effect of $\text{CO}_2/\text{HCO}_3^-$ buffer on pumping rate. Exposure to NH_4Cl causes pH_i to rise because of the influx and subsequent protonation of NH_3 . NH_4^+ is also permeant, however, and its passive influx (the Nernst potential for NH_4^+ is greater than the membrane potential throughout exposure) has an acidifying effect. Thus, on return to normal ASW, pH_i is offset in the acid direction. The cell recovers from this acid load only slowly when HEPES or other non-volatile buffers are used, whereas recovery is greatly accelerated during exposure to a $\text{CO}_2/\text{HCO}_3^-$ buffer at the same pH. The prompt acidification occurring at the onset of the CO_2 exposure (segment BC) is due to the influx, hydration, and subsequent dissociation of CO_2 . Removal of CO_2 (segment DE) causes a reversal of these reactions, and therefore a rapid alkalisation³. The membrane potential in the post- NH_4Cl period was -59 – 60 mV; the Nernst potential for H^+ varied from -56 – 48 . In the experiment in the inset, the pH_o was 7.4 following a similar pretreatment with NH_4Cl -ASW. In HEPES-ASW the pH_i slowly falls (segment AB; rate ~ -0.03 pH h^{-1}) while in $\text{CO}_2/\text{HCO}_3^-$ -ASW, the pH_i rises (segment CD; rate ~ 0.12 pH h^{-1}). During the alkalisation V_m was -62 mV and E_H was -24 – 17 mV. In these experiments, axons were mounted horizontally in a chamber (temperature $= 22^\circ\text{C}$, volume ≈ 0.15 ml) superfused (1 ml min^{-1}) with ASW. CO_2 -containing solutions were delivered from a glass syringe (after equilibration) through CO_2 -impermeable tubing. The glass pH_i electrodes were of Hinké's design⁴.

reversibly blocked by metabolic inhibitors and that an extracellular acidification accompanies the intracellular alkalisation.

These experiments were performed on giant axons of the squid *Loligo pealei*, cannulated at both ends to allow for the introduction of pH and reference electrodes. In the experiment shown in Fig. 1, an axon (pH_i 7.39) was pretreated with artificial seawater (ASW) containing 50 mM NH_4Cl for ~ 30 min. When, after prolonged exposure to NH_4Cl , an axon is returned to ASW, the pH_i reaches a value considerably more acid than the resting pH_i (ref. 3). In the case of Fig. 1, the pH_i fell to 7.03.

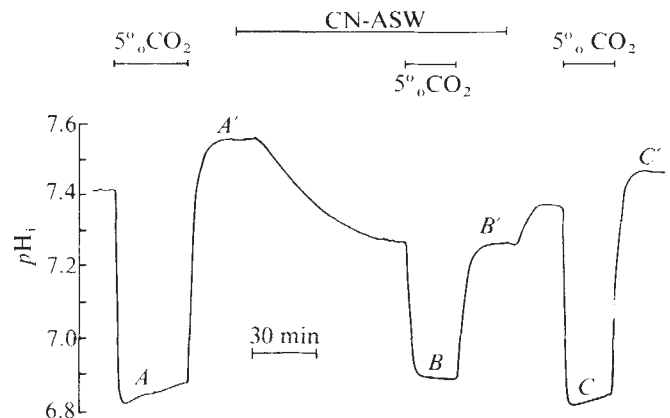


Fig. 2 Secondary alkalisation blocked by cyanide. An axon was first exposed to 5% $\text{CO}_2/50$ mM HCO_3^- -ASW. After the initial acidification, the axon underwent secondary alkalisation (A); on return to normal ASW, the pH_i overshoot the initial pH_i (A'). Subsequent exposure to nominally 2 mM CN caused the pH_i to fall slowly³, and to stabilise eventually over the course of 75 min. Exposure to nominally 5% $\text{CO}_2/50$ mM HCO_3^- , CN-ASW caused the abrupt decline in the pH_i characteristic of CO_2 exposure, but led to no secondary alkalisation (B). Similarly, there was no overshoot of the pH_i when the axon was returned to CN-ASW (B'). After recovery in normal ASW, the axon once again was capable of secondary alkalisation in the presence of CO_2 (C), and exhibited the characteristic overshoot on removal of the CO_2 (C'). The temperature was 22°C throughout.

If a proton pump were designed to maintain a relatively alkaline intracellular milieu in the face of acid challenges, then one would expect the acid load following exposure to NH_4Cl to be an effective stimulus for increased pumping. Indeed, during the time segment AB, the pH_i rose, though very slowly (0.04 pH h^{-1}), when NH_4Cl -free ASW was buffered at pH 8.00 with 0.5 mM HEPES. Replacement of the HEPES buffer with 0.4% $\text{CO}_2/10$ mM HCO_3^- , keeping the seawater pH constant, however, caused the pH_i (after a small acidification due to the influx of CO_2) to rise (segment CD) at a much greater rate (0.35 pH h^{-1}). The stimulation caused by $\text{CO}_2/\text{HCO}_3^-$ at pH_o 7.4 is shown in the inset of Fig. 1. Since in both cases $E_H > V_m$, an active process is required to explain the observed alkalisation. In a series of 11 such experiments, the rate of intracellular alkalisation after the treatment with NH_4Cl was, at a given pH_o , always far greater in $\text{CO}_2/\text{HCO}_3^-$ -ASW than in HEPES-ASW. The rate also increased with increasing pH_o .

The alkalisation occurring in the presence of $\text{CO}_2/\text{HCO}_3^-$ could readily be blocked by the metabolic inhibitors cyanide (CN) and 2,4-dinitrophenol (DNP). Figure 2 illustrates an experiment in which an axon, capable of secondary alkalisation (point A) in the presence of CO_2 , was exposed to CN. After ~ 75 min, when the pH_i had reached a steady level, the axon was exposed to a $\text{CO}_2/\text{HCO}_3^-$ solution in the continued presence of CN. Although typical acidification occurred, no secondary alkalisation was observed (B). Return to cyanide-free ASW and subsequent exposure to CO_2 showed that the axon was once again capable of secondary alkalisation (C). Similar results were obtained with DNP.

Evidence presented so far does not rule out intracellular H^+ sequestration as the cause of secondary alkalisation in the presence of CO_2 . We determined that intracellular secondary alkalisation is accompanied by extracellular acidification, as follows: after pretreatment with 50 mM NH_4Cl -ASW, we placed axons in sealed capillaries containing ASW and phenol red, and followed the absorbance at 559 nm. When the ASW contained 10 mM HCO_3^- (pH_0 8.0), we always observed a decrease in absorbance, corresponding to extracellular acidification; replacing the HCO_3^- with 10 mM TES (pH_0 8.0; pK_a' approximately the same as for CO_2) caused, if anything, an increase in absorbance. Again, treatment with CN prevented the characteristic absorbance decrease in the presence of HCO_3^- . Although the spectrophotometric measurements are difficult to quantify, they provide strong evidence that, as acid disappears from the cell interior, acid appears on the outside.

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Note added in proof: Thomas⁵ has observed that the presumed H^+ pump of snail neurones, too, is more active in the presence of CO_2 .

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The presence of Na–Na and Na–K exchange in sodium extrusion by three species of fish

ALTHOUGH it has been generally assumed that marine teleost fish balance the net influx of NaCl (from diffusional gain and oral ingestion of seawater) by active extrusion of both ions^{1–3}, the proposition of active extrusion of Na has been questioned. It has been shown^{4–11} that marine teleosts generally maintain a transepithelial potential (t.e.p.) in the range +10–+25 mV (blood relative to seawater), which is nearly equal to that which could nullify the Na concentration gradient between the teleost and seawater. Thus, it has been suggested that Na is maintained in electrochemical equilibrium across the teleost gill and only Cl is

actively extruded^{4,6–8,12}. The Na efflux changes accompanying rapid changes in external Na or K concentrations have been used to support a model of both Na–Na and Na–K exchanges^{13–16}, and have been shown to result at least partially from changes in the t.e.p. (refs 7–9, 12) rather than uncoupling of ionic exchange systems. Histochemical localisation of the Na–K-activated ATPase in teleost gill tissue indicates that it is predominantly basal rather than apical^{17,18}. This suggests that the enzyme may be involved in gill cell volume regulation rather than transepithelial extrusion of Na.

Even if marine teleosts maintain blood Na in electrochemical equilibrium with seawater they face a net Na gain because of oral ingestion of the medium^{1,19}. Also, the K-stimulated Na efflux from at least two species of marine teleosts is greater than can be accounted for by changes in the t.e.p. (refs 9, 11). Evans *et al.*¹⁶ have shown that removal of K from the seawater reduces the Na efflux from *Dormitorator maculatus* without affecting the t.e.p. The rapid reduction in Na efflux after transfer of a marine teleost to freshwater (or tap water) is secondary to changes in the t.e.p. (refs 7–9, 12), but Kirschner *et al.*¹² have shown that t.e.p. changes can only account for 60% of the fall in Na efflux from the trout, *Salmo gairdneri*, transferred from seawater to Na-free, K-free artificial seawater.

The validity of the model of Na–K and Na–Na exchange across the marine teleost gill is thus questionable, and to try to describe the components of Na efflux from marine teleost fish more clearly, we investigated the kinetics of Na efflux and the changes in t.e.p. in three species of teleosts, *Achirus lineatus* (lined sole), *Hippocampus erectus* (lined seahorse) and *Opsanus beta* (gulf toadfish) in seawater and other electrolyte solutions. All specimens were collected by us on commercial shrimp trawlers in Biscayne Bay, Florida. They were maintained in seawater (500 mM Na^+) in 30-gallon glass aquaria at $22 \pm 1^\circ C$ for at least 1 week before experimentation and were fed either Tetra (Tetra werke) or live brine shrimp until 24 h before an experiment. The techniques for determination of rapid changes in unidirectional Na efflux and t.e.p. have been described^{9,16}. The only modification was that the t.e.p. was determined on unanaesthetised and submerged sole and toadfish, the seahorse was anaesthetised lightly (4×10^{-5} g per l MS-222) and held with the head and opercula submerged and the site of bridge implantation above the water level. Fluxes and t.e.p. were measured in separate experiments and the fish were either transferred from seawater to tap water or from seawater to K-free, Na-free seawater (–K, –Na SW) and

Table 1 Effect of ion-free solutions on the unidirectional Na efflux and the t.e.p. across three marine teleosts

Species	Na efflux			
	SW	TW	–K, –Na SW	25 K, –Na SW
<i>A. lineatus</i>	100	46 ± 3 (7)*	18 ± 4 (6)*	29 ± 4 (6)†
<i>H. erectus</i>	100	36 ± 1.4 (5)*	19 ± 2 (13)*	27 ± 3 (13)†
<i>O. beta</i>	100	Unstable (10)‡	11 ± 3 (7)	28 ± 2 (7)†
t.e.p.				
<i>A. lineatus</i>	–6.8 ± 0.6 (20)	+5.5 ± 1.4 (6)*	–15 ± 0.6 (5)*	–15 ± 0.9 (5)
<i>H. erectus</i>	–3.5 ± 0.4 (14)	–1.9 ± 1.9 (9)*	–9.2 ± 0.4 (14)*	–9.3 ± 0.7 (14)
<i>O. beta</i>	–8.1 ± 0.9 (12)	Unstable (6)‡	–15 ± 1.5 (6)	–15 ± 1.5 (6)

All data are expressed as mean $\pm$ s.e., number of experiments in parentheses. All flux values are expressed as a % of the control efflux when the animal is in seawater. The t.e.p. is expressed as blood relative to medium (mV).

* Significantly different ($P \leq 0.005$ in paired experiments) from the seawater control.

† Significantly different ($P \leq 0.005$ in paired experiments) from the –K, –Na SW data. –K, –Na SW: 2.5 mM Na^+ , 571 mM Cl^- , 53 mM Mg^{2+} , 10 mM Ca^{2+} , 28 mM SO_4^{2-} , 2.5 mM HCO_3^- , 501 mM choline⁺, 25 K, –Na SW; same as above except that 25 mM K^+ is added as the sulphate salt.

‡ Effluxes and t.e.p. of *O. beta* in tap water are unstable but show the same trends as displayed in the other two species. In tap water the sodium efflux falls to 32 ± 2.7% of the seawater control but within 3–5 min increases to 81 ± 5.5% of the control. In tap water the t.e.p. always depolarises (range –1 to +16 mV) immediately, but within 10 min falls back towards the t.e.p. in seawater and stabilises at a t.e.p. usually somewhat depolarised from the seawater control (range –6 to +6 mV).

then into 25 mM per l K-enriched, Na-free seawater (25 K, -Na SW).

The results are shown in Table 1. All three species maintain their blood distinctly negative to seawater and this is the first description of a marine teleost with t.e.p. blood negative to seawater. Although we did not determine blood Na concentrations in our animals, all marine teleosts have a blood Na concentration well below that of seawater²⁰ so it seems that, unlike other teleosts, *A. lineatus*, *H. erectus* and *O. beta* face a substantial electrochemical gradient for Na across their epithelium. They all display a significant reduction in Na efflux and change in t.e.p. after transfer to tap water. In all cases, however, the t.e.p. becomes less negative rather than more negative as has been described for other teleosts^{7-9,12}. Obviously, changes in the t.e.p. cannot account for the observed reduction in unidirectional Na efflux from these species in tap water. So it seems that the decline in unidirectional Na efflux is secondary to an uncoupling of Na-K and/or Na-Na exchange rather than a change in passive efflux. Tap water, however, is not only Na- and K-free but also free of other ions (for example, Ca, Mg) that may be important in maintenance of the normal marine teleost gill epithelium, and it has been shown that external Ca has profound effects on the fluxes of Na and Cl as well as the t.e.p. in both the freshwater goldfish, *Carassius auratus* and the marine mullet, *Mugil capito*^{21,22}. Thus, it is more relevant to measure the flux and t.e.p. changes after transfer from SW to -K, -Na SW rather than to tap water. In all three species, transfer to -K, -Na SW results in a 6-8-mV increase in the internal negativity and an 80-90% reduction in unidirectional Na efflux. Although the change in the t.e.p. could account for a 10% reduction in Na efflux (see refs 9 and 12 for relevant formulae), it cannot account for the reduction seen experimentally.

The discrepancy between the data in tap water and -K, -Na SW should be noted (especially for *O. beta*). With the exception of that by Kirschner *et al.*¹⁴, other studies of flux and t.e.p. changes in ion-free media have involved the use of tap or freshwater. It can be seen from our work that transfer of a marine teleost to tap water may alter the permeability characteristics of the gill epithelium sufficiently to produce spurious results.

Thus, our data clearly show that the majority of the unidirectional Na efflux from three marine teleosts is coupled to the external K and/or Na concentration exclusive of t.e.p. changes. Data in Table 1 show clearly that, after transfer from -K, -Na SW to 25K, -Na SW, although the t.e.p. does not change, the Na efflux increases in all three species. Thus, Na-K exchange does occur in Na extrusion in these teleosts but (since even 25 mM K l⁻¹ results in an Na efflux of only 30% of the Na efflux in seawater) is a relatively minor component compared with diffusional efflux and Na-Na exchange. In summary, *A. lineatus*, *H. erectus*, and *O. beta* maintain their blood Na out of electrochemical equilibrium with seawater Na and extrude the excess Na by way of an Na-K exchange mechanism. In addition, Na-Na exchange diffusion is important in the unidirectional Na efflux from these species.

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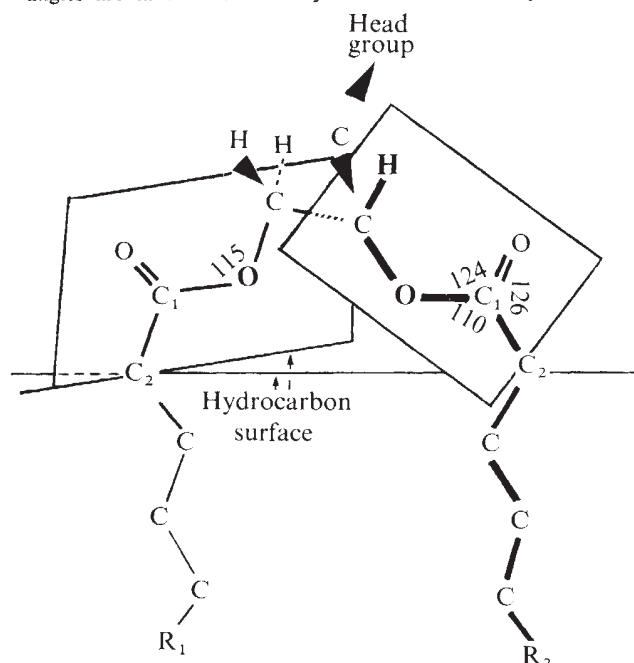
Roles of carbonyl oxygens at the bilayer interface in phospholipid-sterol interaction

FROM many recent studies on the phospholipid-sterol interaction, it has become increasingly clear that the 3 α - and 3 β -hydroxy isomers of a range of sterols have quite different effects on the molecular mobility and functional property of lipid bilayer membranes^{1,2}. For example, the addition of cholesterol or Δ^5 -cholesten-3 β -ol to liposomes decreases their permeability, whereas epicholesterol, a 3 α -isomer of cholesterol, exerts no apparent effect. It has been proposed that the 3 β -hydroxyl group engages in hydrogen bonding with the carbonyl oxygen of the fatty acyl groups in phospholipids in the bilayer³. Evidence supporting such hydrogen bond formation is strong⁴. The problem is, then, why does the 3 β -OH group of cholesterol, but not the 3 α -OH group of epicholesterol form such bonds?

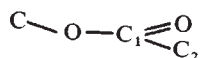
Here I first discuss the molecular configuration of the bilayer interface and its characteristics, and then propose molecular models to describe hetero-intermolecular hydrogen bonds between the carbonyl groups of phospholipids, hydroxyl groups of sterols, and water molecules in the bilayer interface, which enable rationalisation at the difference in activity between the 3 α - and 3 β -hydroxy isomers of sterols.

Fig. 1 Schematic diagram showing the planarity of the structural

element $\text{C}-\text{O}-\text{C}_1=\text{C}_2$ of a phospholipid molecule and the hydrocarbon surface of the bilayer. Values of various bond angles are taken from the crystal structure of acetylcholine⁵.



The small region taken up by the structural element



of phospholipids in the bilayer is termed the bilayer interface. Here, C1 and C2 are, respectively, the first and second carbon atoms of the fatty acyl chain. Several aspects of the geometry of the elements are pertinent. First, a plane passing through all the C2 carbons of the acyl chains can be considered as the hydrocarbon or non-polar surface of the bilayer (Fig. 1). Second, the carbonyl double bond and the partial double bond character of the ester C-O bond cause all carbon and oxygen atoms in the structural element to be coplanar^{5,6}, similar to the atomic arrangements found in the amide plane of polypeptide chains and sphingolipids. Moreover, the C-H bond of the secondary ester is synplanar to C1=O as shown in Fig. 1^{6,7}. Because of this planarity of the structural element, the segmental motions of carbon atoms in the bilayer interface are expected to be rather restricted. Indeed, ¹³C-NMR (nuclear magnetic resonance) experiments demonstrate that the spin-lattice relaxation times of carbon nuclei within the phosphatidylcholine molecule in a bilayer increase from the glycerol backbone to both the methyl ends of the two fatty acyl chains and the -N⁺(Me)₃ end of the polar head group, indicating the smallest segmental mobility of the carbon atoms in the structural element^{8,9}. Finally, on the basis of the planarity of the structural element together with the vertical orientation of the two parallel fatty acyl chains away from the hydrocarbon surface in the liquid crystalline state¹⁰, it is reasonable to assume that the C1=O bond adopts a tilted orientation with respect to the hydrocarbon surface as shown in Fig. 1. In spite of the uncertainty in the precise orientation of the carbonyl groups, it is important to point out that the carbonyl group is held rigidly in proximity to the hydrocarbon phase of the bilayer which has a low dielectric constant.

In discussing the carbonyl oxygen in the bilayer interface serving as a hydrogen bond acceptor, two features of hydrogen bonds must be considered. In the first place, of 196 hydrogen bond angles studied, the configuration of O-H...O bond angles which appeared with the greatest frequency was found to be approximately 15° from the linear configuration^{11,12}. In other words, a stable hydrogen bond has such a configuration that the donor, hydrogen, and acceptor atoms are nearly colinear. Second, the hydrogen bond is electrostatic in origin, with greater strength in a medium of low dielectric constant. The intrapeptide hydrogen bonds, for example, do exist in the interior of proteins where the effective dielectric constant is relatively low.

A plausible model for a phospholipid-cholesterol complex at the interface of a bilayer is presented in Fig. 2a, showing how the equatorial hydroxyl group on C3 can satisfy the necessary conditions for hydrogen bond formation. The rigid, planar fused ring system of the molecule

is inserted vertically into the hydrocarbon core with C3 at the hydrocarbon surface, bringing the more polar hydroxyl group into the interface, where the dielectric constant is still sufficiently low to enhance the formation of a hydrogen bond.

It is not likely that water can displace and thus compete with cholesterol for the formation of stable hydrogen bonds to carbonyl oxygens at the same binding site as shown in Fig. 2a, because the necessary orientation would bring water molecules near the hydrocarbon phase, an energetically improbable circumstance. Water molecules may, however, form bonds by orientating themselves in solution towards the polar surface of the bilayer. This possibility would be favoured by the presence of cholesterol, which increases the separation of head groups on the phospholipid molecules¹³, and therefore also increases the configurational freedom of water molecules in the upper portion of the bilayer interface. Thus, my model predicts that no more than one molecule of water will form hydrogen bonds with each carbonyl oxygen atom of the bilayer (two per molecule of phospholipid) in any event, and bonding may be even more difficult in the absence of cholesterol.

The model explains why sterols with the 3 α configuration lack activity in membrane functions. Figure 2b illustrates the orientation of epicholesterol, the 3 α isomer of cholesterol, when it is positioned so as to bring the hydroxyl group into the interface; Fig. 2b shows that the O-H...O bond angle is approximately 55°, too great for effective hydrogen bonding¹². Lowering the sterol so as to bring carbon 3 to the same level as that shown for cholesterol has the effect of not only increasing the distance between the OH group and the carbonyl oxygen which is unfavourable for hydrogen bond formation, but also partially embedding the hydroxyl oxygen atom into the hydrocarbon region of the bilayer which is energetically unfavourable.

In summary, I believe the key difference between the effect of cholesterol and epicholesterol on the bilayer structure and function lies in their hydrogen-bonding abilities toward the carbonyl group of the ester linkage of the acyl side chains of phospholipids. This difference is a natural consequence of the constrained orientation of these carbonyl oxygen atoms. It is well known that the polar head group of cholesterol molecule may dictate the orientation of the whole molecule in the monolayer¹⁴. I would like to point out that the hydrogen-bonded structural effect of cholesterol may also lead to long range effects on the packing density of the hydrocarbon chains of neighbouring phospholipids. Such changes in hydrocarbon packing would be expected to affect the permeability properties of the bilayer membrane¹. Furthermore, a simple substitution of the hydrogen bond donor from cholesterol shown in Fig. 2a to proteins affords a reasonable extension of the molecular model to explain some of the lipid-protein interaction. In fact, ¹³C chemical shifts of carbonyl groups of different classes of lipoproteins have been reported¹⁵. These observed chemical shifts are consistent with a molecular model in which carbonyl oxygens of lipids are engaged in hydrogen bonding to water molecules and other membrane components³.

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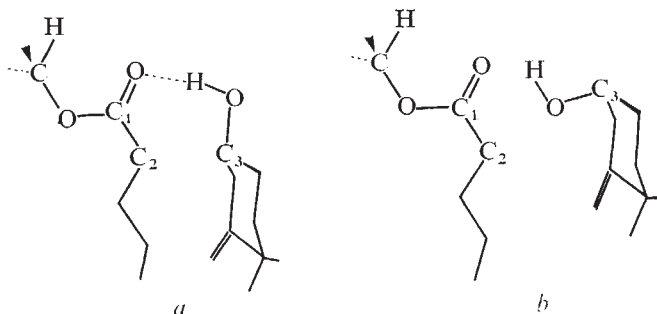
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Fig. 2 Plausible spatial orientation of cholesterol (a) and epicholesterol (b) in the bilayer interface. Hydrogen-bond formation between the phospholipid carbonyl group and cholesterol hydroxyl group is given in (a) as a dashed line.



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Effect of prostaglandin synthesis on renal function and renin in the dog

THERE is considerable evidence for an interrelationship between the prostaglandin and renin–angiotensin systems in the kidney. Infusion of angiotensin II into the renal arteries of dogs and rabbits releases a vasodilator prostaglandin-like material into the renal vein^{1–4}. It also seems that prostaglandins (PGs) induce renin release in the kidney. Riley and his coworkers^{5,6} infused PGE₁ in to the dog renal artery and observed increases in plasma renin activity (PRA) of renal vein blood and in renin secretion. Elevated PRA was observed in blood from the inferior vena cava, just above the entrance of the renal vein, after aortic infusions of PGE₁ (ref. 7) and infusion of PGE₁ and PGA₁ in to the stenosed renal arteries of hypertensive dogs, caused an elevation of renal vein renin⁸. PGE₂ was shown to stimulate renin release in a rabbit renal cortex cell suspension⁹. Indomethacin decreased the PRA of normal and hypertensive rabbits^{10,11}, and prevented the elevation of PRA in rabbits with glycerol-induced acute renal failure¹². In contrast, however, Vander¹³ infused both PGE₁ and PGE₂ into the dog renal artery and failed to observe an effect on renin output.

More recently, Larsson *et al.*¹¹ infused sodium arachidonate, the precursor of PGE₂ and PGF_{2α}, into the descending aorta of the rabbit and observed elevated PRA in blood from the

inferior vena cava. Their control PRA levels were, however, abnormally high (which they attributed to the anaesthetic), and neither renin secretion, urinary parameters, nor renal vein PG levels were measured during the sodium arachidonate infusion. Anggard and Larsson¹⁴ stimulated renin release by the addition of arachidonic acid to an *in vitro* rabbit kidney preparation. In our study we examined the effects of sodium arachidonate-induced changes in renal function on renin output.

The increased renal blood flow (RBF) caused by the infusion of sodium arachidonate is shown in Table 1. There were no significant changes in the mean glomerular filtration rates (GFR) of the three infusion periods or the GFR of the three urine collections in the sodium arachidonate period. Since the GFR was not altered, the calculated filtration fraction decreased, suggesting predominantly efferent arteriolar dilatation. The increase in RBF occurred in the absence of any systemic effects and was characterised by a delay in onset of 31.5 ± 18.2 s (s.e.m., $N = 11$). Coincidental with the increase in RBF there was a marked diuresis and natriuresis. In the first control period the urinary flow rate and sodium excretion rate for the right kidney were lower than those of the left. This is probably the result of surgical trauma, because the right kidney was unavoidably manipulated and the left kidney was untouched. The contralateral kidney demonstrated no significant alterations during the infusion of sodium arachidonate. The haemodynamic, diuretic and natriuretic responses are similar to those reported by others who infused PGE₂ and sodium arachidonate into dog renal arteries^{15,16}. Accompanying the urinary and RBF changes were significant increases in renal vein PRA, renin secretion and renal vein PGF material. The PGF levels were used as indicators of increased activity of the arachidonate–prostaglandin system, since sodium arachidonate has been shown to stimulate synthesis and release of both PGE and PGF by the kidney^{15,17}.

All responses to sodium arachidonate infusion were markedly attenuated by pretreatment with indomethacin. The inhibition by indomethacin of the sodium arachidonate-induced elevation

Table 1 Effects of sodium arachidonate on renal function in dogs

Infusion periods	$\dot{V}$ ($\mu\text{l min}^{-1} \text{g}^{-1}$)				$U\dot{V}_{\text{Na}^+}$ ($\mu\text{Eq min}^{-1} \text{g}^{-1}$)				FF		RBF ($\text{ml min}^{-1} \text{g}^{-1}$)		Renal vein PRA ($\text{ng ml}^{-1} \text{h}^{-1}$)		Renin secretion ($\text{ng min}^{-1} \text{h}^{-1}$)		Renal vein PGF (pg ml^{-1})	
	L (16)	R (16)	L (7) PI	R (7) PI	L (16)	R (16)	L (7) PI	R (7) PI	R (16)	R (7) PI	R (16)	R (7) PI	R (10)	R (7) PI	R (6)	R (5) PI	R (7)	R (5) PI
Control	41 ± 6	33 ± 6	16 ± 5	15 ± 4	3.0 ± 0.4	1.9 ± 0.4	1.2 ± 0.5	1.1 ± 0.4	0.27 ± 0.03	0.32 ± 0.03	4.1 ± 0.4	4.9 ± 0.5	4.2 ± 0.6	2.1 ± 0.4	94.3 ± 32.8	73.8 ± 29.9	181 ± 60	200 ± 22
Sodium arachidonate	40 ± 6	51* ± 7	12 ± 3	19 ± 6	3.2 ± 0.5	3.3* ± 0.5	1.3 ± 0.3	1.7 ± 0.6	0.19* ± 0.02	0.31 ± 0.03	5.8* ± 0.5	5.1 ± 0.5	7.6† ± 1.3	2.6 ± 0.6	473.8‡ ± 107.5	100.9 ± 48.7	443‡ ± 123	268 ± 87
Control	34 ± 5	32 ± 4	13 ± 3	11 ± 3	3.4 ± 0.5	2.5 ± 0.4	1.4 ± 0.3	1.2 ± 0.4	0.27 ± 0.03	0.28 ± 0.01	4.0 ± 0.4	4.8 ± 0.3	3.9 ± 0.6	3.3 ± 0.8	101.1 ± 46.1	132.0 ± 64.3	229 ± 109	178 ± 58

The effects on renal function of sodium arachidonate in salt-loaded dogs and salt-loaded dogs pretreated with indomethacin (PI). Values are given as the mean $\pm$ s.e.m. Number in parentheses are the number of animals. R, Right (infused) kidney; L, left (control) kidney; $\dot{V}$, urinary flow rate; $U\dot{V}_{\text{Na}^+}$, urinary sodium excretion rate; FF, filtration fraction; * $P < 0.001$; † $P < 0.01$; ‡ $P < 0.05$. Twenty-three female mongrel dogs were fasted 18 h before surgery and allowed free access to water. Anaesthesia was induced by intravenous pentobarbital. Indwelling polyethylene catheters were inserted into the right and left femoral arteries for monitoring systemic blood pressure and collecting blood samples, respectively. A catheter was also placed in the left femoral vein and advanced into the right renal vein. A fourth catheter was placed in the right femoral vein for the infusion of inulin. A suprapubic midline abdominal incision was made and both ureters cannulated. A right retroperitoneal flank incision was made, and a pneumatic cuff and electromagnetic flowprobe were placed around the renal artery. The pneumatic cuff was used to establish and verify the electronic zero flow. RBF was monitored by a sine wave, non-occluding electromagnetic flowmeter (Biotronex Laboratories). A 25 gauge infusion needle was inserted into the right renal artery, normal saline was infused at 0.5 ml min^{-1} . The animals received an intravenous saline load of 10% body weight, after which the intravenous saline infusion was matched to the urine output of 0.5 – 2.0 ml min^{-1} . Seven animals received an intravenous injection of indomethacin at a dose of 10 mg kg^{-1} buffered to pH 7.4. One hour was allowed before the commencement of the experiment. The study was comprised of three 30-min periods; three 10-min urine collections were obtained during each period. Midway through each period arterial and renal vein samples were collected for inulin, sodium, renin and PGF determinations. After the first control period, sodium arachidonate²⁸ (Nu Chek Prep) was infused into the renal artery, delivering $25 \mu\text{g per kg body weight per min}$. Thirty minutes was allowed for renal function to return to control levels and then the final control period was begun. The animals were killed and the kidneys removed and weighed. Renin and PGF levels were determined by radioimmunoassay. The level of significance was determined by the paired Student's *t* test.

in renin secretion indicates that sodium arachidonate is not active *per se* but must be converted to another compound, such as one of the intermediary endoperoxides, thromboxane A_2 and/or PGs.

There are several possible explanations for the observed increase in renin release. Both renal nerve stimulation and the infusion of catecholamines into the renal circulation enhance renin release^{18,19}, and PGF has been shown to potentiate adrenergic activity^{20,21}. Frame *et al.*^{22,23}, however, observed that PGE₂ and sodium arachidonate attenuate renal sympathetic transmitter release and vascular responses to noradrenaline in the rabbit kidney. Therefore, it is doubtful that the sympatho-adrenal system is involved in the sodium arachidonate-induced renin release. Riley *et al.*⁵, concluded that the PGE₁-induced elevation of renin output was due to vasodilatation and physical distortion of the juxtaglomerular apparatus. Tagawa and Vander²⁴, however, found that vasodilatation induced by acetylcholine had no effect on renin release. Furthermore, Witty *et al.*²⁵ observed no significant alterations in renin secretion when papaverine, a potent vasodilator, was infused into the renal artery of the denervated non-filtering kidney, the denervated filtering kidney and the innervated non-filtering kidney of the dog. This suggests that renal vasodilatation or juxtaglomerular apparatus distortion do not necessarily result in increases in renin secretion. In addition, several investigators have shown that decreases in renal perfusion pressure which result in decreased afferent arteriolar stretch stimulate renin secretion^{26,27}. Werning *et al.*⁷ and Varkarakis *et al.*⁸ attributed the stimulation of the renin-angiotensin system by PGE₁ to alterations in the sodium concentration in the tubular urine acting through the macula densa. In contrast, Tagawa and Vander²⁴ demonstrated that acute water and electrolyte losses induced by renal vasodilatation did not result in elevations in renin secretion. These observations indicate that distortion of the juxtaglomerular region, changes in sodium flux at the macula densa or adrenergic stimulation are not primarily responsible for the increase in renin release seen with the infusion of sodium arachidonate. PGE₂ and arachidonate stimulation of renin release in the *in vitro* rabbit kidney preparation also argue against these theories. We, therefore, favour the concept that the arachidonate-PG system stimulates renin secretion by a direct action on the juxtaglomerular apparatus in the intact animal.

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Interaction of tryptophan transfer RNA with Rous sarcoma virus 35S RNA

THE primer for the initiation of DNA synthesis by the RNA-directed DNA polymerase (reverse transcriptase) on Rous sarcoma virus (RSV) 70S RNA has been identified as tryptophan tRNA^{1,2}. The RNA primer hybridises efficiently to 35S RNA subunits produced by denaturation of 70S RNA from RSV³ or avian myeloblastosis virus (AMV)⁴; these template-primer complexes are active in the initiation of DNA synthesis by reverse transcriptase³⁻⁵.

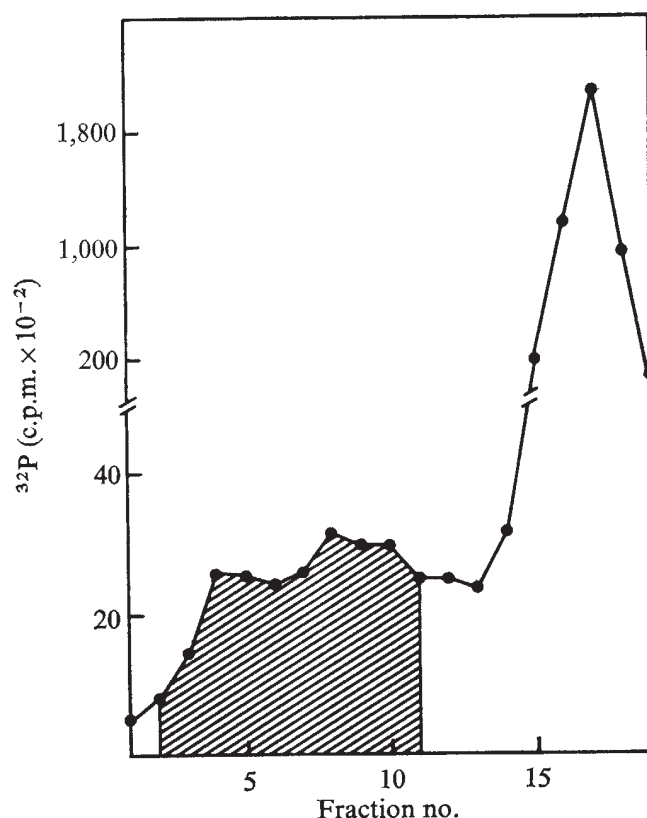


Fig. 1 Isolation of 35S RNA-tRNA^{Trp} hybrids by sucrose density gradient centrifugation. Prague C RSV 35S RNA (125 µg) was hybridised with ³²P-tRNA^{Trp} (26 µg; 70,000 c.p.m. µg⁻¹) according to the procedure of Waters *et al.*⁴. The hybridisation mixture was subjected to centrifugation in a 15-30% (w/v) sucrose density gradient as described previously¹². The radioactivity in each fraction was monitored by Cerenkov radiation and the fractions comprising the high molecular weight RNA region (shaded area) were pooled and precipitated with ethanol¹². RSV RNA was prepared as described previously¹². Uniformly labelled ³²P-tRNA^{Trp} was isolated by two-dimensional electrophoresis¹³ from the free 4S RNA fraction of ³²P-labelled RSV. The identity and purity of tRNA^{Trp} was established by fingerprint analysis (see Fig. 3a). Non-radioactive RSV (Prague C) was obtained through the courtesy of Dr R. E. Smith from University Laboratories, Highland Park, New Jersey, under the auspices of the Office of Program Resources and Logistics, National Cancer Institute.

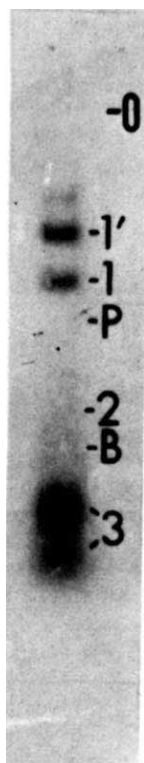


Fig. 2 Autoradiogram of the polyacrylamide gel fractionation of a 35S RNA-tRNA^{Trp} hybrid RNase T₁ digest. P, Position of undigested tRNA^{Trp} determined from a parallel electrophoretic run; B, position of bromophenol blue tracking dye; O, origin. Electrophoresis was from top to bottom. RNA hybrids, prepared as described in the legend of Fig. 1, were dissolved in 100 μ l of 0.01 M Tris-HCl, pH 7.5-0.1 M NaCl. RNase T₁ (Sankyo Co., Japan; obtained from Calbiochem Corp.) was added to give a final concentration of 0.83 U per 100 μ g RNA. After 30 min at 37 °C, the digest was rapidly cooled, diluted with two volumes of electrophoresis buffer (0.045 M Tris, 1.4 mM EDTA, 0.045 M boric acid, pH 8.3) and subjected to electrophoresis in a 10% polyacrylamide gel¹³. Those areas of the gel containing ³²P-RNA were excised and the RNA eluted as described previously¹¹. In the case of small oligonucleotides (area 3), the gel was crushed and suspended in electrophoresis buffer. The radioactive material was then recovered by electrophoresis on to DEAE paper⁷.

We have examined the nature and extent of the interaction between tRNA^{Trp} and RSV 35S RNA by characterising fragments of tRNA^{Trp} which result from limited digestion of 35S RNA-tRNA^{Trp} hybrids with RNase T₁. The large, undigested fragments of tRNA^{Trp} represent regions of the polynucleotide chain which are complementary to the 35S RNA. Earlier studies on bacteriophage RNAs have shown that, in the appropriate conditions, guanylate residues normally susceptible to the action of RNase T₁ are resistant to digestion when base paired in regions of secondary structure⁶⁻⁸.

³²P-tRNA^{Trp} was hybridised to non-radioactive 35S RNA. The resulting hybrids were separated from uncomplexed tRNA^{Trp} by sucrose density gradient centrifugation (Fig. 1). After recovery by ethanol precipitation, the hybrids were dissolved in a buffer of high ionic strength to stabilise the interaction between the polynucleotide chains, then digested with RNase T₁. The digestion products were fractionated by polyacrylamide gel electrophoresis (Fig. 2).

Digestion of the 35S RNA-tRNA^{Trp} complex resulted in the release of two components which moved more slowly than free, undigested tRNA^{Trp} (bands 1' and 1, Fig. 2) and smaller components which had mobilities greater than tRNA^{Trp} (band 3, Fig. 2). In some digests an additional component was often present; although not apparent in the digest represented in Fig. 2, its position of migration is indicated as band 2. When unhybridised tRNA^{Trp} was digested in the presence of 35S RNA using the same digestion conditions used for the 35S RNA-tRNA^{Trp} complex, radioactive material was detectable in the position of band 3, but not in the positions of bands 1', 1 or 2.

The radioactive fragments comprising bands 1', 1 and 2 were completely digested with RNase T₁ and the products of digestion were fractionated either by one- or two-dimensional electrophoresis. Figure 3 shows the fractionation patterns obtained for bands 1', 1, and tRNA^{Trp}. The fractionation pattern obtained for band 2 was identical to that of band 1; band 2 presumably results from partial dissociation of the double-stranded RNA complex (band 1) during dilution in the electrophoresis buffer before electrophoresis.

The oligonucleotides resolved by electrophoresis were identified by comparing their pancreatic RNase digestion products with the pancreatic RNase digestion products of the oligonucleotides obtained from the complete RNase T₁ digestion of intact tRNA^{Trp}.

Since the nucleotide sequence of tRNA^{Trp} has been determined¹, it is possible to determine from which region of the tRNA the oligonucleotides released by RNase T₁ were derived. All the oligonucleotides present in bands 1', 1, and 2 are derived from the 3' end of the molecule (Fig. 4). This region of the tRNA must therefore be complementary to the 35S RNA. The analysis of material in the area of the

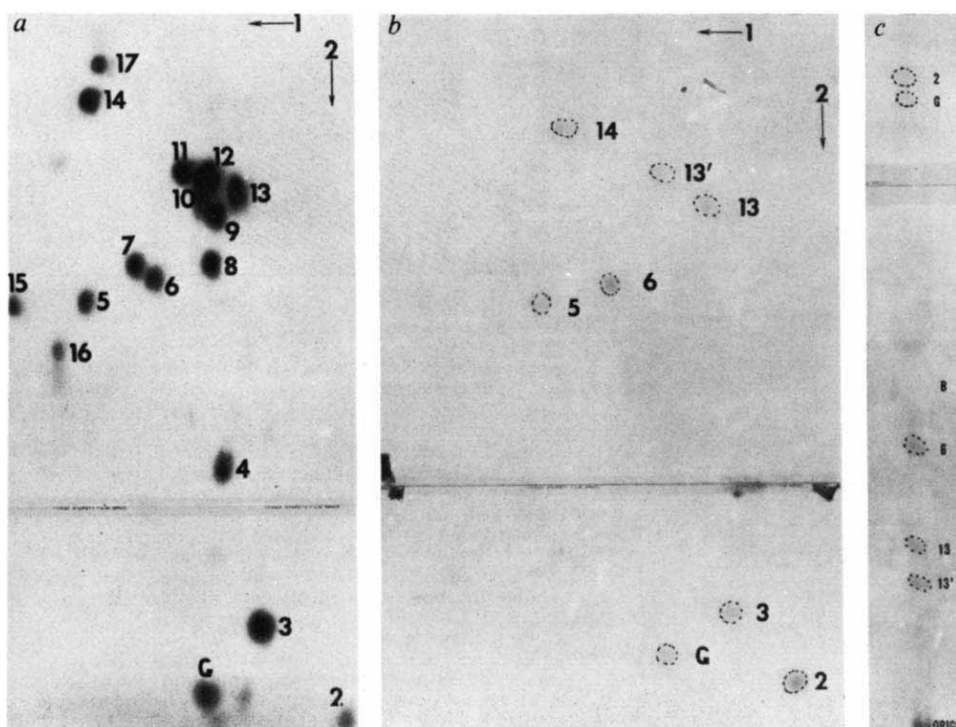


Fig. 3 Electrophoretic separation of complete RNase T₁ digests of fragments of 35S RNA-tRNA^{Trp} hybrid complexes. *a* and *b*, Fractionation^{14,15} of digests of tRNA^{Trp} (control) and band 1', respectively. 1, Direction of electrophoresis in the first dimension; 2, direction of electrophoresis in the second dimension. *c*, One-dimensional fractionation on DEAE paper of a digest of band 1. B, Position of the xylene cyanol blue marker; G, guanosine 3'-phosphate. The oligonucleotide numbering system is identical to the system of Faras *et al.*^{1,16}. Oligonucleotide 13' results from the rearrangement of m⁴A to m⁶A in oligonucleotide 13.

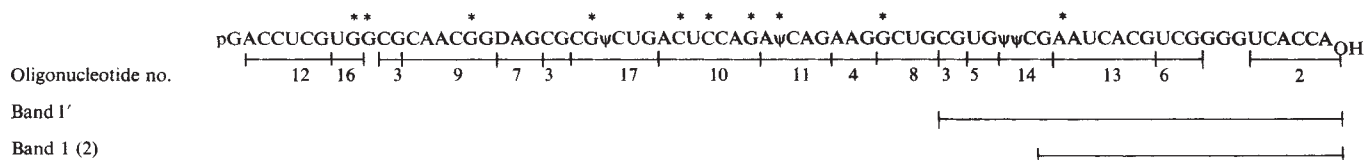


Fig. 4 tRNA^{Trp} fragments resistant to RNase T₁ digestion in 35S RNA-tRNA^{Trp} complexes. The nucleotide sequence of tRNA^{Trp} and the numbering system of the oligonucleotides have been described previously^{1,16}. *, Modified nucleotides. Solid lines under a nucleotide sequence indicate regions protected from digestion with RNase T₁.

gel designated as band 3 showed only the presence of oligonucleotides derived from the 5' end of the tRNA (data not shown).

The finding that the 3' end of the tRNA^{Trp} molecule is complementary to the 35S RNA is not surprising in view of the fact that polymerisation by reverse transcriptase results in the covalent linkage between the terminal 3'-OH adenosine of the primer RNA and the first deoxyadenosine residue of the nascent DNA⁹⁻¹¹.

It seems likely that the interaction of tRNA^{Trp} with viral RNA would still allow for the formation of both the anticodon and dihydro U loops as well as the maintenance of the anticodon arm portion of the 'L'-shaped tertiary conformation¹⁷. In addition, the 3' end of the tRNA may interact with the viral RNA so as to maintain the overall symmetry of the tRNA. The symmetry of the complex might be important with respect to its recognition by reverse transcriptase¹⁸.

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11-*cis* vitamin A in dark-adapted rod outer segments is a probable source of prosthetic groups for rhodopsin biosynthesis

THROUGHOUT life, the outer segments of rod photoreceptors (ROS) are renewed continually¹, a process that entails

assembly of new disk membranes at the base of the ROS. Biosynthesis of the apoprotein of rhodopsin, the major membrane protein of the ROS disks, is known to occur in several stages in the inner segment²⁻⁵. Bok has found, however, that completion of the rhodopsin molecule by combination with its retinaldehyde prosthetic group does not take place until after the apoprotein has been transported to the ROS⁶, perhaps just before its insertion in to the membrane. The process continues unimpaired in darkness^{7,8}, so that in these conditions a pool of 11-*cis* vitamin A should be readily available close to the site of synthesis.

Frogs (*Rana pipiens*, southern variety) were dark adapted for 24 h at 25 °C. The retinae were cleanly dissected in Ringer solution, any small areas with adhering pigment epithelium being cut away and discarded. Wherever possible, all subsequent operations were carried out in a nitrogen atmosphere with nitrogen-purged solvents. The retinal material was separated into two fractions by syringing through a No. 17 trochar to shear off the ROS and centrifuging on a discontinuous sucrose gradient according to Papermaster and Dreyer⁹. The ROS were quantitatively collected and pelleted in water. Attempts at further purification always resulted in appreciable loss of vitamin A. The residual ROS-denuded retina was washed with water. Subsequent rhodopsin extractions with 2% digitonin indicated that 90% of the ROS had been removed by this procedure.

The tissue fractions were freeze-dried and powdered thoroughly, and the vitamin A extracted by grinding three times with aliquots of light petroleum (b.p. 37-48 °C). A further small quantity of vitamin A could be extracted if the residues were mixed with water, methanol and then light petroleum, but it amounted to only 8-10% of the total. The methanol treatment destroys rhodopsin, however, so appreciable amounts of retinaldehyde were also present.

It was found that the ROS contained a small, but measurable, supply of vitamin A and that it differed markedly in both isomeric configuration and degree of esterification from that found elsewhere in the retina. After filtration (Millipore FHLPO 1300) and concentration to equal volumes, the light petroleum extracts were chromatographed by high pressure liquid chromatography (HPLC; see legend to Fig. 1 for details)¹⁰. Retinyl esters eluted first and were followed several minutes later by retinol isomers. The separated ester and alcohol fractions were quantitatively transferred to 0.1 ml of dry chloroform. One drop of acetic anhydride was added, followed by 0.9 ml of Carr-Price reagent (saturated solution of SbCl₃ in chloroform). Spectra of the blue colour generated by this reaction are shown in Fig. 1a and b. The λ_{max} are at 620 nm, and the absorbance depends on the amount of vitamin A present, irrespective of its isomeric configuration and whether it is esterified or not¹¹.

The ROS-denuded retina accounted for about half of the total vitamin A, most of it (82%) being in the form of ester. On the other hand, the alcohol predominated (82%) in the ROS, which distinguished them not only from the rest of the retina but also from the pigment epithelium^{10,12,13}. The vitamin A in this tissue is almost entirely esterified in the yellow oil droplets and 92% in the cytoplasm¹⁴.

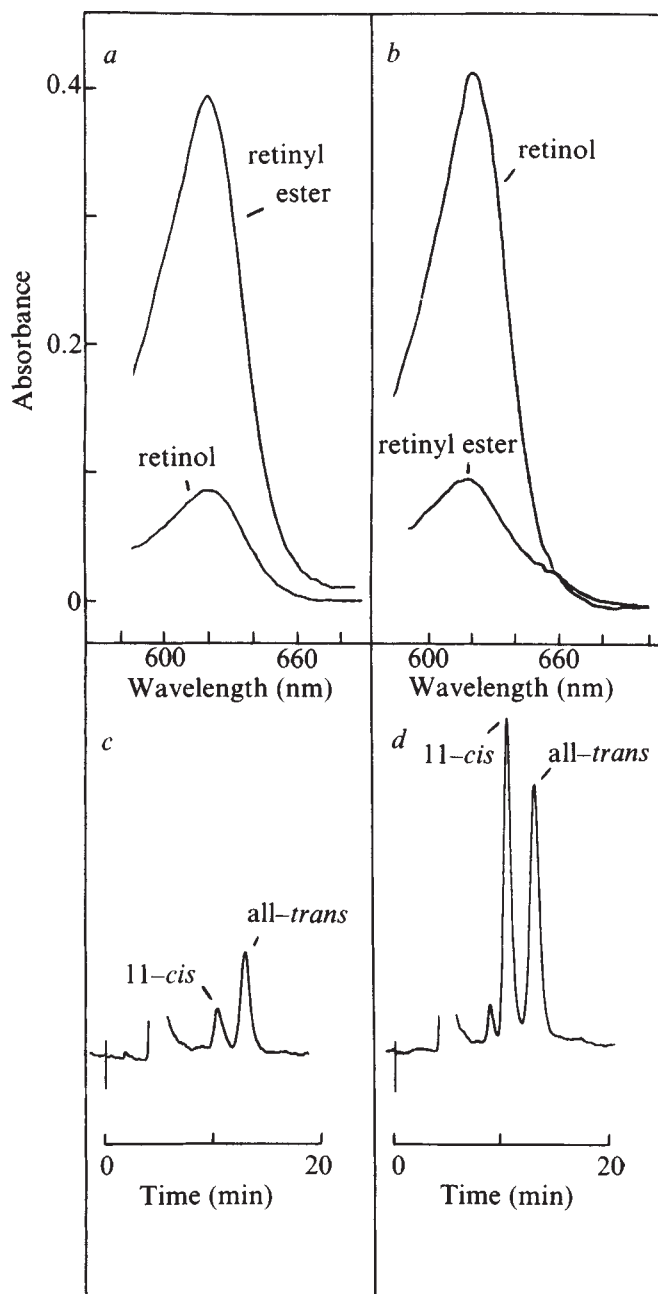


Fig. 1 Vitamin A content of ROS compared with the rest of the retina. *a* and *b* show the spectra of the Carr-Price "blues" (Cary 14 spectrophotometer) from the retinyl ester and retinol fractions separated by HPLC as summarised below. Both colour intensity and $\lambda_{\max}$ are independent of isomer configuration and whether alcohol or ester forms are involved¹¹. The ROS contained 3.69 nmol vitamin A of which only 0.69 nmol was ester. From the rest of the retina the yield was 3.48 nmol, of which 2.86 was ester. *c* and *d* show the HPLC records obtained. Retinyl esters eluted between 3.5 and 7.0 min. Other absorbing substances, including small amounts of carotenoid esters, appeared during this time but did not interfere with the Carr-Price reaction. For clarity, the initial peak of the HPLC record has been blocked out. The 11-*cis* and all-*trans* retinols eluted between 9.5 and 16.0 min. The minor peak preceding 11-*cis* in the ROS extract was not identified; its size varied between different experiments. The experimental conditions were: Columns, 2 m $\times$ 2 mm stainless steel packed with Corasil II (Waters Associates); eluant, 1% isopropanol in hexane (Fisher H-291); flow rate, 0.9 ml min⁻¹ from a Waters model 6000 pump; detection, 310 nm at absorbance 0.05 setting on an Instrument Specialties model UA-5 monitor; injection, 24 μ l, Chromatronix fixed-loop valve. Recovery of retinol from the column averaged 95%, retinyl ester 100%. *a*, *c*, Retina without ROS; *b*, *d*, ROS.

As Fig. 1*b* and *c* shows, the alcohol fractions eluted in two bands maximal at 10.6 and 13.0 min identified respectively as 11-*cis* and all-*trans* retinol¹⁰. The 11-*cis* isomer accounted for as much as 60% of the ROS retinol, but the all-*trans* isomer preponderated in the small quantity of retinol present in the remainder of the retina.

The vitamin A content of the ROS amounts to 4.2 mol % of the rhodopsin, determined by digitonin extraction of the light petroleum-extracted residues. This corresponds to about a 2-d supply of prosthetic groups if ROS growth proceeds at the rate of $\sim 2\%$ d⁻¹ (ref. 1). The amount of 11-*cis* retinol alone is thus sufficient to sustain rhodopsin renewal for about 1 d. Normally, the pool of ROS retinol would be continually replenished, probably from supplies in the pigment epithelium which are known to amount to about 2 mol of retinyl ester per mol of retinal rhodopsin¹⁰. The ester could be converted to alcohol before transfer to the retina, but it is more likely that it is transported unchanged and hydrolysed in the ROS. The latter cannot synthesise ester, although other parts of the retina can¹⁴⁻¹⁶. The derivative of vitamin A that is converted to 11-*cis*, and where this isomerisation takes place in the eye, is being investigated and will be discussed elsewhere.

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Corrigendum

In the article "Mutation rate, genome size and their relation to the rec concept" by J. A. Heddle and K. Athanasiou (*Nature*, **258**, 359; 1975) the following corrections should be made. Page 360, line 5: the value of *m* should be 1.68×10^{-18} ; line 6: the *b* and *r* values should be 0.91 and 0.98, respectively. In Table 2 the genome size of ϕ X174 and S13 should be 1.7×10^6 .

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matters arising

Chinese cosmology

GRIBBIN'S essay¹ is based on a few pages of Joseph Needham's *Science and Civilisation in China*², but makes an exaggerated claim for Chinese openmindedness as contrasted with Western narrowness by disregarding Needham's sensitivity to context and implications, and by painting an extremely one-sided picture of the European tradition. The statement that Western science was a branch of religion three hundred years ago is either misinformed or extremely ill-stated. Gribbin confuses cosmology and astronomy when he talks about the centre of development of the science shifting to the West by the end of the seventeenth century. "The science" must mean astronomy, mentioned in the preceding sentence. It is impossible to sustain an argument that the centre of astronomy lay in China until that time. Needham proves that early Chinese astronomy was as estimable as that of the West, but his argument ignores the consistent superiority of Europe in computational astronomy. Gribbin is apparently unaware of the work of Nakayama³, myself⁴, and others who have provided independent quantitative comparisons in the nearly twenty years since volume 3 of *Science and Civilisation in China* was completed.

Gribbin's article reads like a caricature of Needham, because he ignores everything that does not fit his thesis. One could make an equally one-sided argument in the other direction by noting that Aristarchus of Samos, in the third century BC, asserted that the Earth revolves about its own axis and about the Sun, an idea that never appeared independently in China. Aristarchus also suggested that the stars were immeasurably far from the Sun, but Gribbin does not mention this.

Although it is true, as Gribbin says, that any study of Chinese astrology and cosmology must draw on Needham's work, it cannot end there. Needham's sections on astronomy and mathematics depend much more heavily on the secondary literature than his later volumes. His accounts of early cosmology require correction from other sources. The earliest concept did not, as Needham and Gribbin claim, represent the heavens as a hemispherical dome. As Nakayama has

shown in English, there were two stages; in the first sky and Earth were flat, and in the second they were indefinitely vault-shaped. The second concept was not that of "a celestial sphere surrounding the spherical Earth". Recent Chinese research has proved what Western writers have suggested, namely that the Earth in this model was either flat or hemispherical⁵. Neither concept, in fact, was a cosmology either in the modern sense or in the sense that gave philosophical cosmology authority over computational astronomy in the early West. The two Chinese concepts did not displace each other because it was a matter of no practical consequence which was correct. The independence of astronomy from cosmology in China has been noted by many recent writers. Of the several ancient authorities that Needham cites regarding the "empty space" theory, only one was an astronomer.

Gribbin's reading on supernovae also inadequately represents the very considerable literature that has accumulated⁶. He does not mention the best-documented supernova of all, that of 1006, and does not seem to be aware that Ho *et al.* have produced evidence to cast considerable doubt on his identification of the supernova of 1054 with the Crab Nebula⁷.

Now let me take up what I consider ill advised about Gribbin's topic itself. Historians of science have by now given up treating ancient speculations as foreshadowings, forerunners, or anticipations of the precise concepts of the modern exact sciences, since no one who has hunted for such anticipations with sufficient determination (and willingness to abide bad metaphors) has failed to track them back to the dawn of history. Instead historians think of early ideas as constituents in an endlessly complex and never predictable development from one phase of understanding and practice to the next. I am unable, in any case, to understand why Gribbin believes the Mohist idea that motion requires duration is equivalent to any innovation of Einstein. He also finds deep significance in the fact that the Chinese language includes a compound in which one character stands for space (in the everyday sense) and one stands for time, but this is a purely lexical matter, and has nothing at all to do

with the modern physical concept which we express inadequately in quotidian language as "a continuum in four dimensions".

To sum up, Dr Gribbin has not taken the trouble that he assuredly would have done had he prepared a scientific paper for *Nature*. He has not studied enough, and has not taken sufficient pains to understand what he has studied.

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Moinian and Lewisian of Sutherland and the Morarian Orogeny

IN view of speculation concerning the Moinian geology of Sutherland^{1,2}, a short resume of our research in central Sutherland (S.J.M.) and the Bettyhill–Strathy area of north-eastern Sutherland (V.E.H.) is of interest.

In central Sutherland there are three large, sub-parallel Lewisian sheets within the Moinian west of the migmatite complex³. In the Bettyhill–Strathy area there are sheets and broad areas of Lewisian. Our extensive geochemical data indicate that the majority of the Lewisian in these areas has strong Scourian affinities^{4–6}. The Borgie Lewisian⁶ and that at Ribigill near Tongue (unpublished), contains both Scourian and Laxfordian types. Geochemical distinctions between the western and the eastern Moinian in northern Sutherland⁷, cited by Garson and Plant¹, represent a distinction between Moinian with extensive basic Lewisian in the west, the Tongue–Borgie area, and acid Lewisian with

some Moinian in the east, the Armadale-Strathay area.

Garson and Plant¹ and Johnson² mention ultrabasic bodies intruding the Moinian and the Lewisian of Sutherland, as originally described by Read³. Our detailed field mapping has shown them to be restricted to the Lewisian. Read⁴ and the later authors^{1,2} apparently confuse a series of Lewisian metaperidotites and garnet pyroxene rocks (garnet pyroclastics) with early Moinian metabasic intrusives. Geochemical and petrological evidence indicates that the garnet pyroxene rocks of the Naver and Borgia⁵ Lewisian are identical to those of the foreland Lewisian^{9,10}. Similarly, the metaperidotites are identical to analogous rocks found throughout the foreland Lewisian^{8,10,11}. These bodies are not, therefore, synchronous with the emplacement of the Lewisian sheets (see ref. 2), they are an integral part of them. Neither are they high level diapirs (see ref. 2); nor do they constitute a Moinian ophiolite zone (see ref. 1).

Several authors have speculated an extension of the Sgurr Beag Slide¹² into central Sutherland^{2,13,14}. Although slides are present there is no definite evidence that any are continuous with the Sgurr Beag Slide. The Lewisian sheets of central Sutherland seem to have been emplaced by a combination of early (F1) isoclinal folding and possible sliding, which has been modified by later (F2) folding and sliding. The continuation of the Sgurr Beag Slide may, however, occur in north-eastern Sutherland, thus extending Johnson's speculation², the Lewisian of central and northern Sutherland may be the root zone of Moravian nappes represented by the large areas of Lewisian in north-eastern Sutherland.

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Evolutionary changes in insulin

BLUNDELL and Wood¹ propose "a model for the evolution of insulin mainly in terms of adaptive processes". Simultaneously they raise questions regarding "conclusions made on the basis of uniform rates and changes that seem not to affect function", such conclusions being attributed to Kimura² and to King and Jukes³. They do not mention the more recent discussion by Kimura and Ohta⁴, who point out that insulin in general has a very low evolutionary rate, corresponding to a strong negative-selection barrier. Blundell and Wood's model calls for functional restraints on most of the amino acid residues. Kimura and Ohta also noted that the C peptide of proinsulin evolves about 10 times as fast as insulin, and is therefore a much better candidate for neutral mutations than insulin itself, and they proposed that the absence of zinc from guinea pig insulin results in a loss of constraint on the fixation of mutations.

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Specificity of transfer factor

IN an article on the use of transfer factor (TF) in treatment of viral infections¹, Zuckerman states that "clinically, TF activates uncommitted and non-sensitized lymphocytes of the recipient so that new clones of antigen-specific cells are produced". He goes on to make the point that the delayed-type skin reactivities that are transferred after injection of TF are those possessed by the donor. It is undoubtedly true that the results obtained during the past 25 yr by Lawrence and others are most readily explained by the existence of such specificity². The problem is, however, that nobody has found a way in which specificity can be demonstrated in unequivocal terms and by procedures which allow for confirmation in other laboratories. At the recent Second International Workshop on Basic Properties and Clinical Applications of Transfer Factor at Fort Detrick, there were more than 100 participants and not one convincing demonstration of specificity.

Failure to convince does not disprove the concept of antigen specificity, but it is worrying. It may be due to the difficulties of human experimentation and the lack of a satisfactory animal

model. One possibility is that TF preparations contain both a specific factor and nonspecific adjuvant-like factors, and that this is creating problems in man where the antigens normally studied are not complete strangers to the 'negative' donors and recipients. The various *in vitro* systems that have been used have not pointed to specificity, although there is no guarantee that the factors active *in vivo* are being assayed. The work of one of us has involved the action of human TF on macrophage migration and lymphocyte transformation^{3,4}. Evidence obtained in the latter system suggests that the action of TF is nonspecific but that the level of this nonspecific activity is greater in tuberculin-positive than in negative individuals.

Doubts about specificity are especially relevant in the clinical field where there is clear evidence that TF can boost phytohaemagglutinin responsiveness and the number of cells forming rosettes with sheep erythrocytes^{5,6}. This nonspecific action could be responsible for the appearance of delayed hypersensitivity and clinical improvement.

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Insectivorous grouse?

BEFORE embarking on the "full-scale investigation of the relationship between grouse and insects" recommended by your Animal Ecology Correspondent¹, the cautious research worker might wish to establish that incubating hen red grouse do in fact eat many tipulids. This is crucial to the suggestion that hen grouse on wet moors may rely on tipulids to redress their supposed state of 'nutrient depletion' after egg laying. The work by Butterfield and Coulson² did not show that adult hen grouse eat tipulids at this stage, although it would not be surprising if readers of their paper were to infer that they do.

Although Butterfield and Coulson present data for the month of May, when hens are incubating, the droppings that they collected in May were from cock grouse. How do we know this? When incubating, hen grouse

produce large, distinctive 'clocker' droppings, rather like those of broody chickens. Before their paper was submitted, we asked Dr Coulson if clocker droppings had been included in the May sample, and we also described what they look like. He said they had noticed some droppings like that, but discarded them as they did not know what the droppings were.

If tipulids do 'play a vital part in the maintenance of grouse populations', as suggested by your Animal Ecology Correspondent we should expect wet, blanket-bog grouse moors where there are many tipulids to support more grouse than drier moors. In fact, the reverse is the case. The wettest moors have the fewest grouse. Contrast western Ireland where the extensive, wet bogland supports good populations of tipulids (A. N. Lance, personal communication) but very few grouse³ with the typically dry moors in the north-east of Scotland where grouse are abundant⁴. Almost all good grouse moors are on dry ground and the association between dry ground and good grouse shooting is so widely understood that draining wet ground to increase grouse numbers is an accepted management practice.

The reason for this is clear. Heather covers a greater proportion of the ground and grows best on dry moors, and numbers of grouse are correlated both with the proportion of ground covered by heather and with the vigour of its growth⁵.

The nutritional requirements of an adult grouse are highest when the hen is laying eggs. It is this time when nutrients are most likely to be limiting and when insect food is most likely to be an important dietary supplement. Laying hen grouse do not eat many insects, because insects are not abundant this early in the season. The suggestion remains that hens become depleted of nutrients while laying eggs and that insect food, especially tipulids, is essential during the incubation period in order to redress this hypothetical imbalance. It is not claimed that this occurs on dry moors, because adult grouse eat few insects on dry moors. The suggestion is limited to wet moors.

This limitation implies that there must be some difference between the diets of laying hens on wet and dry moors, such that hens on wet moors are less well fed than on dry moors. This is possible because one important determinant of the hens' plane of nutrition in spring is the density of heather available⁶; and heather tends to be sparser on wet moors⁷. Therefore we agree that it would be wise to improve the hens' plane of nutrition in spring.

It is most helpful to improve the

food for the hens before they begin to lay eggs. When the laying hens' food is good, the chicks which hatch survive better⁸ as a result of improvement in the quality of the eggs⁸. The food can be improved by proper heather management, which includes draining on wet moors and results in the provision of a dense array of heather shoots. The alternative suggestion, mooted by your Animal Ecology Correspondent, is that tipulids should be provided after the hen has laid her eggs. This procedure can have no effect on the quality of the eggs.

There is no evidence that hen grouse do eat tipulids during incubation. Even if they do, there is no evidence that tipulids are ever in short supply on wet moors. The only observed association between tipulid densities and grouse numbers is that grouse stocks are higher where there are fewer tipulids—on dry moors.

We do not attempt to argue that tipulids are totally unimportant on wet moors, because there is no direct evidence one way or the other. But we do suggest that heather will remain much more important for grouse numbers than tipulids, even if the 'nutrient depletion' hypothesis proves correct.

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Temperature, phenoxybenzamine and adrenoceptor transformation

IN his letter¹ Benfey suggests that the adrenoceptor transformation hypothesis is incorrect because temperature-induced changes in the block by and binding of phenoxybenzamine (POB) in frog hearts² could result from a change in the rate of alkylation of nonspecific nucleophilic centres. The impression is given that the hypothesis is based only on the observed effects of POB; however, several reports indicate that the competitive α -adrenoceptor antagonist, phentolamine, can also effectively inhibit inotropic and chronotropic responses to catecholamines in the hearts of various species at low temperature³⁻⁷. In addition, tem-

perature is not the only factor that has been shown to alter the properties of adrenoceptors. Cardiac inotropic and chronotropic responses in hypothyroid rats are affected by POB and phentolamine at high temperatures similarly to those of normal hearts at low temperatures⁸⁻¹⁰. These observations clearly cannot be due to an effect of temperature on nonspecific alkylation. A nonspecific temperature effect might explain Benfey's observation¹ that POB blocks acetylcholine more effectively at higher temperatures. This temperature effect, however, is opposite to that observed with cardiac adrenoceptors, and parallels the increase in POB binding we have found in homogenised myocardium. Protection from POB block by high concentrations of membrane stabilisers does not prove that α -adrenoceptors are not involved. Local anaesthetics can influence the apparent affinity of an antagonist with α -adrenoceptors¹¹.

The concentration of POB required to block responses to adrenaline in frog hearts at low temperature is clearly higher than that required to block α -adrenoceptors in vascular smooth muscle, however, α -adrenoceptors are probably not all identical. Relatively high (micromolar) concentrations of either POB or phentolamine are necessary to inhibit presynaptic α -receptors in the heart^{12,13} and all but one study demonstrating an α -adrenoceptor component of inotropic responses of mammalian hearts have used high concentrations of α -blocking drugs^{8,10,14-17}. The only claim that nanomolar concentrations of POB or phentolamine can inhibit responses to phenylephrine in rabbit left atria¹⁸ is disputed¹⁹. Lack of complete block with even high concentrations of α -adrenoceptor antagonists could also reflect the fact that no 'pure' cardiac α -adrenoceptor agonist is available, and the considerable residual β activity at low temperature, or in hypothyroidism, could 'mask' α -adrenoceptor block.

The absence of a temperature-induced difference in the blocking action of propranolol or in the relative potencies of agonists reported by Benfey is contrary to observations from several laboratories³⁻⁷, and the reason for the difference is not clear. His data do, however, suggest that propranolol is more effective in hearts that are more responsive to β -adrenoceptor stimulation. The standard errors in his Table 1 indicate up to 50-fold variation in sensitivity to isoproterenol. Propranolol greatly reduced this variability, showing a greater effect on the more sensitive preparations. Benfey does not indicate the temperature during exposure to propranolol: incubation at 24 °C before testing the block at 14 °C could account for the lack of a decrease in inhibition.

It is unlikely that the previously reported effect of propranolol at low temperature³⁻⁷ was due to inadequate diffusion of the drug. Benfey found that atropine produced the same block at low and high temperatures after 10-min incubation. Hypothyroidism also reduced the inhibitory effect of propranolol, although all tests were at the same temperature and after 30-40-min incubation⁸⁻¹⁰.

In conclusion, Benfey's data seem to be inadequate to support his contention that the hypothesis of adrenoceptor transformation is an artefact attributable to nonspecific alkylation by POB.

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BENFEY REPLIES—The above comment¹ on my letter² misses the point. The real issue is not whether a change of temperature alters the rate of alkylation by phenoxybenzamine (although it obviously does), it is not how much phenoxybenzamine is required to block α -adrenoceptors (although Kunos *et al.*³ loaded the tissue by four 10-min exposures to 2.5 μ g ml⁻¹ of the highly lipid-soluble drug), and it has nothing to do with hypothyroid rats (although it may be noted that mammals have cardiac α -adrenoceptors in normal conditions at normal temperature⁴). The issue is whether lowering temperature transforms the adrenoceptor of the frog heart from its characteristic β -type into an α -receptor. If this were so, one might reasonably expect that phenylephrine would be more potent at a low than at a high temperature,

that the reverse would be true for isoprenaline, and that the blocking ability of propranolol might be changed by lowering temperature. These predictions were not supported by my experiments, and this was the reason for not accepting the hypothesis that the nature of the adrenoceptor changes with temperature⁵.

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Aedes aegypti complex in Africa

We wish to discuss aspects of the communication by Scott and McClelland¹. It was pointed out long ago that sound ecological studies depend on the accurate determination of the evolutionary status of the organisms under study². This is especially crucial in applied biology³. If two forms are common and sympatric there are three possible situations. (1) They are reproductively isolated and do not exchange genes under natural conditions; in other words they are distinct species. If they are not reproductively isolated (that is they mate with each other in nature), they may represent (2) two subspecies (races) whose ranges have recently intersected to form a transient area of sympatry where hybridisation occurs, or (3) morphs of a single polymorphic species⁴. The concepts behind these possibilities are well established and backed by sound bodies of theory, and many studied examples of each are documented⁵.

Scott and McClelland dealt with the sympatric occurrence of the dark and pale forms of the current taxon *Aedes aegypti* L., a mosquito of considerable medical importance. The pale form has a wide distribution within and beyond Africa, and is domestic in its habits. The dark form is confined to Africa south of the Sahara, and is an outdoor mosquito⁶. These facts and the data on the alkaline phosphatase and protein loci provided in Table 1, of ref. 1, are sufficient to eliminate the possibility that we are dealing with a single polymorphic species. In fact, the data for these two loci provide strong evidence for the existence of positive assortative mating in the field, thus supporting the view that they are distinct genetic species. It should be emphasised that the evidence for random mating in cages and for the interfertility of the two forms is of little relevance in assessing the status of the forms because it is well established that distinct genetic species are often interfertile

in artificial crosses^{7,8}, and cases are known where distinct species mate at random in cages^{7,9} but positively assortatively in nature^{7,10,11}.

We think that it is unfortunate that Scott and McClelland used the vague and imprecise terms "ecotype" and "incipient species". "Ecotype" is a term introduced for plants and is not appropriate to animals. The term "incipient species" is not appropriate here, as it is usually used in narration to imply some well marked subspecific stage. Neither term is backed by a satisfactory body of theory. The opening sentence of the report immediately leads to confusion by implying that "ecotypes" are not populations.

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SCOTT AND MCCLELLAND REPLY—We agree that the status of the two entities is crucial to the paper. We believe that field hybridisation has been amply demonstrated (refs 4 and 9 of our report in *Nature*¹). A computer simulation of this situation containing three genotypes, three habitats, two seasons, parameters for rate of movement, habitat selection, natural selection and assortative mating, is now under review. It demonstrates that polymorphism is highly probable, provided only that: (1) there is a dry season when breeding occurs only in the indoor habitat; (2) fitness of the indoor ecotype is greater in houses, and fitness of the outdoor ecotype is greater in the natural habitat; and (3) movement between indoor and outdoor habitats is not large (less than about 25% of the indoor inhabitants must be immigrants each generation). The computer model shows that, when mating is random, polymorphism is probable.

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reviews

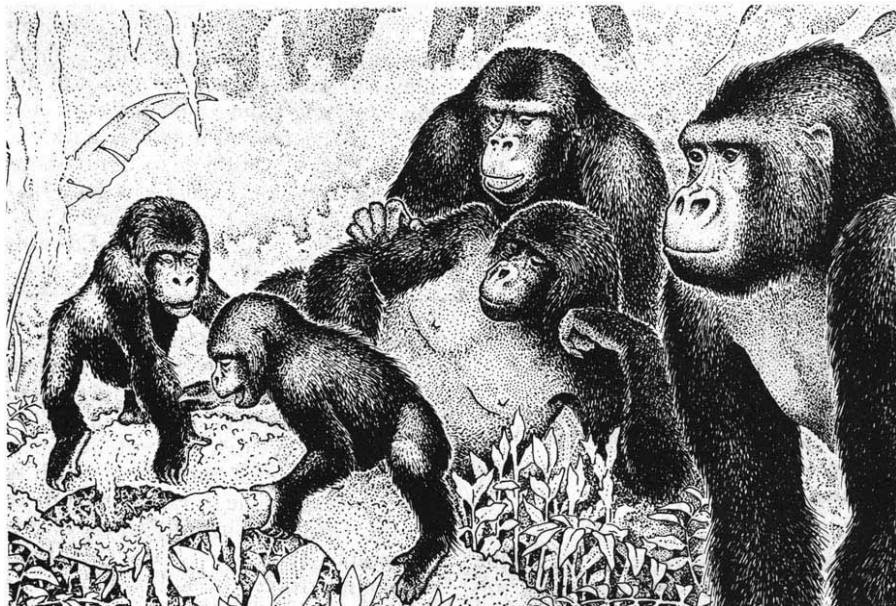
Bull's-eye of sociality

THIS is a book* addressed to a wide audience and one likely to make a lasting impact on biological thought, research and teaching. Its title puts a new name to a subject that is among the fastest-growing areas of biology, and its contents do indeed provide a new synthesis, of wide perspective and great authority.

Dr E. O. Wilson is one of the best-known zoologists of the day, and collaborated with the late Robert MacArthur in writing the biological best-seller, *Island Biogeography* (1967). Since then he has produced, among other works, a large, universally acclaimed monograph on *The Insect Societies* (1971). His output is extraordinary: *Sociobiology* itself contains about half a million words. His plain uncluttered prose is a treat to read, his logic is rigorous, his arguments (even the occasional mathematics) are lucid. He is a real polymath, threading a constructive path through forests of zoological literature and, as far as one can tell, rarely putting a foot wrong. In a difficult and controversial field, his deductions are informed, balanced and fair, even though some are necessarily questionable.

The book is an evolutionary study of social biology throughout the animal world, or more exactly, throughout the Metazoa. It is divided into three sections of which the first, 'Social Evolution' is the shortest, and deals among other subjects with the fundamentals of sociality, population biology, and selection processes. The second, Social Mechanisms, covers communication, dominance and territorial systems, parental care, and much else. The third is entitled The Social Species, and in 200 pages provides a critical review of more than 30 selected groups, chosen from the invertebrates, cold-blooded vertebrates, birds, mammals and primates (including man). The coverage and digestion of the literature are amazing: about 2,500 references are listed, making the bibliography a goldmine in its own right. At £11 the book is a bargain.

**Sociobiology: The New Synthesis*. By Edward O. Wilson. Pp. 697. (Belknap Press of Harvard University: Cambridge, Massachusetts, 1975.) £11.



I am no stranger to the perplexities of sociobiology; they have occupied much of my time for over 20 years. Having read the book through I find myself wondering how close Professor Wilson has come to the bull's-eye, in pondering what sociality is and does, as an evolutionary adaptation. The basic target is not easily perceived, perhaps because the adaptive value of sociality is different, at least superficially, for different animals. The simple definition he gives of a society (p7) is "a group of individuals belonging to the same species and organised in a cooperative manner". He stresses (p379), as being among the key properties of social existence, cohesiveness, altruism and cooperativeness; and often, especially in reviewing the social species, he contrasts social with solitary animals, as if flocking or sociability were also a key property. Thus he affirms (p484) that sociality has been advanced in those African mammals that have forsaken the closed forests and become adapted to open grasslands. But we know that there are many kinds of fish, birds and mammals that vary their flocking strategy from day to day and hour to hour, sometimes in fact depending on whether they are in open or closed habitats. Can the distinction between clumped and solitary dispersion indeed be fundamental?

He sees four pinnacles of social evolution, found in (1) the colonial invertebrates, (2) the social insects, (3) the vertebrates and (4) man himself, and he observes that the first three

have been scaled many times by independently evolving lines of animals, although man has scaled his peak on his own. In the context of Professor Wilson's special group, the social insects, he picks out as their particular social traits, the cooperation of individuals in the care of the young, the presence of more or less sterile individuals working on behalf of fecund nestmates, and an overlap of at least two generations capable of contributing to colony labour.

It is perhaps here that he comes nearest to identifying two other properties that seem to me to lie still closer to the bull's-eye of sociality: first, the potential endurance of animal societies, so that they characteristically outlive their current members; and second, their property of imposing duties and restraints on their members, serving to promote the common good, often in the longer term. Selection for altruism itself is admirably discussed.

He regards those animals, the colonial invertebrates, that have scaled the first pinnacle as coming close to producing perfect societies; and, looking towards the other three pinnacles, he asks why the overall trend has been downward from this primitive or simple perfection. The siphonophores are to him "the *ne plus ultra*"; whereas from my different point of view it is hard to concede that they qualify as societies at all. They are of course composite or compound animals, made up of variously modified polyps, and they use budding as a means of growth and organ differentiation; but each siphono-

phore complex develops from a single zygote into a ciliated larva, and later into a primordial polyp, forming, as it grows, a stem from which the other polyps branch. After a reproductive period its planktonic existence ends, and it dies as an entity. Perhaps it is not in every sense one individual; but can its state of organisation properly be equated with that of the higher societies on any of the other pinnacles—societies composed of separate, potentially competing, genetically different, sentient individuals, bonded together by sensory recognition and appetite, constrained by conventions

and by traditions handed down from forbears to descendants as the generations pass. Once more I must doubt it, and whether indeed the author could claim (which of course he does not) yet to have found the keys to this black box.

Ultimately these questions are important, but for the present I think they will worry most readers not at all, nor detract from the pleasure and profit to be found in this remarkable book. They do nothing to diminish its new horizons, but remind us only that we are not at the end of the road.

V. C. Wynne-Edwards

Superconductivity

The Superconducting State. (Graduate Student Series in Physics.) By A. D. C. Grassie. Pp. vii+135. (For Sussex University Press; Chatto and Windus: London, September 1975.) £8.00.

THE author, in his preface, excuses the appearance of yet another book on superconductivity by claiming that "It corresponds to the level of the experimental physicist who needs to follow theoretical papers in order to analyse his results adequately . . .". Having on many occasions suffered restrictions imposed by a lack of theoretical understanding, I approached this volume with eager anticipation. It is with regret that I report that after reading, and indeed re-reading, the book my hopes remain unfulfilled.

The first chapter, an introduction to the phenomena of superconductivity, sets the historical perspective. The second, dealing with the microscopic Bardeen, Cooper and Schrieffer (BCS) theory of superconductivity requires fairly advanced quantum mechanics, and will severely tax most experimental physicists. A less mathematical treatment of BCS is probably not possible, but a closer attempt to relate formulae to physical reality would be helpful. The strong-coupling extension of BCS, which is needed to discuss the technically interesting, high critical temperature superconductors, is dismissed in two paragraphs. The limitations of the theory, for example in its ability to predict new superconductors, are ignored.

The following two chapters cover the Ginsburg-Landau phenomenological theory, and the magnetic state of type II superconductors. The treatment is on a par with those to be found elsewhere, and offers no new insight. The latter section is incomplete in that it ignores the temperature dependence of κ .

The reader is entitled to expect more technological bias from chapter 5, en-

titled "The Current-Carrying Capacity of type II Superconductors". The main reason for using superconductors in large electrical devices is their ability to sustain very high current densities, but the pinning of flux lines, which is directly responsible for the magnitude of the critical current, receives one paragraph. It is true that "the origin of pinning forces is still a subject of detailed research", but then so are many of the other phenomena described. There is sufficient agreement among workers interested in flux-pinning for the author to have given some indication of the physical nature of pinning forces. The discussion of flux instabilities is better, though in one of the all too rare numerical calculations the author arrives at the incorrect value of 0.042 cm for the critical half-thickness of a stable conductor. Using the author's formulae and quoted values for parameters, the correct answer should be 0.003 cm, a value which looks less out of place with the accompanying photograph of a multifilamentary conductor.

The final chapter is concerned with Josephson effects, and the devices which use them. This is the best chapter in the book, not only because it is the most original. But at six pence a page, the book must be regarded as expensive.

David Dew-Hughes

Insect biochemistry

Insect Biochemistry and Function. Edited by D. J. Candy and B. A. Kilby. Pp. xii+314. (Chapman and Hall: London, May 1975. Distributed in USA by Halsted Press.) £8.50.

THOSE expecting a comprehensive textbook of insect biochemistry will be disappointed. This book is in fact a collection of four review essays describing the biochemistry of certain functions in detail, and the authors assume the reader to have a good basic knowledge of biochemistry. The

greater part of the book is devoted to aspects of insect flight, with one chapter on the utilisation, and another on the sources of fuels for flight. The first describes the metabolic pathways for the release of energy from carbohydrates, fats and amino acids. Emphasis is placed on the complex interactions resulting in the breakdown of muscle glycogen as an immediate source of energy, the importance of certain metabolites (especially α -glycerol phosphate, proline and acetyl carnitine) for penetrating mitochondria, and the mechanisms responsible for controlling mitochondrial metabolism, which may increase by as much as 100 fold on the initiation of flight. The nature of these fuels for flight, and the sites for their storage are described belatedly in the second chapter. The importance of the various energy sources for both short and long periods of flight are discussed, and attention is drawn to the central role of the fat body in synthesising and storing these fuels.

The diversity and origins of excretory materials produced by insects is the subject of the third essay which dispels the firmly entrenched generalisation that insects are primarily uric acid excreting organisms. Many nitrogenous compounds are excreted, including ammonia which, despite its great toxicity, is now recognised as a major excretory product in some terrestrial insects. The accumulation of uric acid within the insect body has until recently been thought of as excretion, but evidence is reviewed that this metabolite is mobilised in times of dietary stress, possibly by intracellular symbionts within the fat body.

The final contribution is a concise assessment of our current knowledge of synaptic transmission in insects. After a general outline of the structure of the insect nervous system, cholinergic transmission is summarised very briefly, and the remainder of the chapter is devoted to a discussion of the evidence for glutamic acid, γ -amino butyric acid and biogenic amines as excitatory and inhibitory transmitters both peripherally and in the central nervous system.

Although the editors draw attention to the importance of comparative aspects of biochemistry in achieving selective toxicity in chemicals used for pest control, little emphasis is placed on these, even in the most relevant chapter on synaptic transmission. The book provides, however, good reviews of the three topics, and these have comprehensive bibliographies (the chapter on excretion being especially notable in this respect). It should prove useful reading for both undergraduates and research workers in insect biochemistry. A. L. Devonshire

Tool not a discipline

Enzyme Kinetics: Behaviour and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems. By Irwin H. Segal. Pp xxii+957. (Wiley-Interscience: New York and London, July 1975.) £15.00.

ONE might have expected a book on enzyme kinetics by I. H. Segal to make as useful a contribution to its field as his *Biochemical Calculations* has made within its own category of elementary textbooks. Unfortunately, this has proved otherwise. His new volume is a long and detailed account of just one aspect of enzyme kinetics: steady state methods. Yet it is possible to read through its excess of 900 pages and at the end of it not to have learnt anything about the behaviour of enzymes. The rationale of the study of the kinetics of enzyme reactions is surely the information that kinetic methods provide about the operation of enzymes, either from the viewpoint of the elucidation of the chemical mechanism of the enzyme or the understanding of some biological phenomena. In my opinion, enzyme kinetics is a tool of biochemical research, not an intellectual discipline within its own right. This book presents the latter view.

The heart of the book is a catalogue of steady-state rate equations for enzyme mechanism containing one, two or three substrates reacting with the enzyme in all possible sequences together with the inhibition patterns expected for different types of inhibitors. The mathematics have been presented clearly and double-reciprocal plots of reaction velocity against reactant concentration illustrate most of the equations. No examples of the use of these equations in enzymology are given, which is regrettable because the value of a method can only be judged by the problems it solves. The nomenclature of enzyme mechanisms used throughout the book is that of Cleland. But the principles of steady state kinetics were established more than 20 years ago by Haldane, Alberty and Dalziel, who demonstrated when these methods could distinguish between alternative reaction mechanisms and, equally important when they could not. The newer nomenclature of Cleland is useful on account of its systematic approach but yields no new principles nor unifying concepts. The same comment might apply to this book. The effort here has been expended on description rather than comprehension. For instance, it has never seemed to me helpful to use terms such as Bi Uni Uni Ping Pong Ter Bi to denote a reaction mechanism since they fail to advance an understanding of the mechanism at the physical level.

Therefore, this book is recommended only to professionals working in the field of steady state enzyme kinetics. Though the derivation of the rate equations for any single mechanism is tedious rather than difficult, some will find it useful to have available a compendium of steady state equations for a variety of different systems derived for them. But the restriction to a single experimental method treated in a purely theoretical manner renders the book unsuitable for the general student of biochemistry. One can admire the rigour with which the author approaches his subject but not the basic premise from which he starts.

S. E. Halford

Breath of air

Principles of Comparative Respiratory Physiology. By Pierre Dejours. Pp. xvi + 253. (North Holland: Amsterdam and Oxford; American Elsevier: New York, 1975.) Dfl. 35; \$14.75.

HAVING an almost exclusively neurophysiological interest in respiratory movements, I undertook the reviewing of *Principles of Respiratory Physiology* with some trepidation, since I knew this would force me to tread very unfamiliar paths through the jungle of work on respiratory gas exchange in different animals. I am glad to say that my fears were unfounded. True Frenchman that he is, Dr Dejours has created a masterful 'Guide Bleu' through this jungle, providing a clear trail for the timorous, yet offering the more adventuresome rich local detail and source references on which to linger.

I particularly like the way in which he deals with the relevant physical principles of diffusional and convective gas flows, the O₂ and CO₂ capacitances of the gaseous and fluid compartments of the respiratory system and their corresponding O₂ and CO₂ conductances. The operation of these principles in the living organism is illustrated throughout the book by appropriate reference to phylogenetic or species specific mechanisms of gas exchange in both water and air breathers representing the most lowly and most complex of animals. The net result of this endeavour is that Dejours has produced an impressive, overall view of gas exchange even for the most complex Metazoa in which he traces the flow of O₂ from the environment to the mitochondrion by way of the alveolar gas, gas-blood barrier, blood, systemic capillary walls, interstitial fluids and cell membrane, together with the flow of CO₂ in the reverse direction.

The clear authority of the author is no better marked than in the closing chapter where he expresses the view point that the principal aim in the

control of respiration is the adequate oxygenation of the tissues in contrast to the more commonly held view that it is the CO₂ pressure and acid-base balance which are primarily regulated. His defence rests on the evidence that only long term changes in the needs of oxygenation lead to significant adaptive morphological, biochemical and physiological changes of the organism.

Highly recommended to all air breathers interested in respiration.

T. A. Sears

Electronic structure

Electron Densities in Molecules and Molecular Orbitals. (Physical Chemistry: Series of Monographs, Volume 35.) By John R. Van Wazer and Ilyas Absar. Pp. ix+101. (Academic: New York and London, September 1975.) \$14.50; £7.25.

THIS is an unusual book. Essentially the authors have a computer programme for Hartree Fock electronic wave function calculations for molecules incorporating flexible possibilities for the basis sets and a perspective three-dimensional graphic output expressing the electron density throughout a cross-sectional plane. This they use for total and for one-electron densities for LiF, H₂O, HCP, C₂H₆, PF₃O and some 30 other molecules. The book largely presents these graphics and associate text for undergraduate chemists "whose minds are spatially oriented"; for the more expert there is little except possible illustrations for a lecture.

A greater attention to detail is necessary, such as clear bold labelling of the molecule for each figure, clear indications of the nuclear positions, clearer depiction of nodal lines, omission of slapdash jargon—such as reference to the C₃ axis of PHF₂O—and careful spatial orientation of all figures relating to the same molecule such as 3.1 and 3.2 on page 29. Emphasis on the shape of the electron density in a single cross section downgrades the importance of kinetic energy, ignores spin effects, ignores time correlation features associated with configuration interaction and leaves the weaker student with a dominant picture of molecular orbitals as sharp peaks on each nucleus (where ψ^2 remains large for s orbitals) surrounded by a trough (associated with the relatively unimportant spherical nodal surface in 2 s orbitals) and a few small bumps associated with bonds.

Others may agree that there are better ways of introducing simple electronic structure to undergraduates.

D. H. Whiffen

obituary

Professor Thomas A. Bennet-Clark, CBE, FRS died peacefully after a long illness on November 24, 1975. He was born in Edinburgh in 1903 and educated at Marlborough College and Trinity College, Cambridge, where, under the inspiration of F. F. Blackman, he switched from Chemistry to Botany, and won the Frank Smart prize (1923). He started research under Blackman but after a year moved to Trinity College, Dublin, to become research assistant to H. H. Dixon. From 1930 to his retirement in 1967, he held University posts in Manchester, Nottingham, Kings College, London and East Anglia.

Blackman's influence on Bennet-Clark can be seen in his early contribution to plant metabolism, but he went beyond the essentially analytical work of Blackman. Whereas Blackman's analysis of the Pasteur effect in apples made no reference to foreign research, Bennet-Clark was quick to recognise the deep significance of Meyerhof's work on muscles. Bennet-Clark's work on metabolism was a landmark and initiated a series of investigations leading from those of Meirion Thomas on CO_2 fixation to the present recognition of the underlying biochemical unity in the organic acid metabolism of succulents and C_4 plants.

The six years spent with Dixon were particularly happy years. The work at Dublin on cell membranes and permeability led to a study of osmotic relations. This work raised the possibility of the active uptake of water, which excited much interest and inspired a great deal of experimentation in plant biophysics.

Bennet-Clark adapted filter paper chromatography for the assay of activators and inhibitors of plant growth. This work initiated an important series of investigations on plant hormones particularly involving the work of Wain and Wareing. His work on diageotropism (in collaboration with Sir Nigel Ball) did not lead to major developments in plant physiology. The experiments do, however, reveal great ingenuity and originality, and should be read by all young plant physiologists.

Bennet-Clark's contribution to Plant Physiology was highly original but its quantity was not large. Partly this was due to his high regard for quality, partly due to limited facilities, but most of all due to his unselfishness. He was full of ideas, his quick mind produced lateral thoughts at an alarming rate, few, however, were tested experimentally because he would not use his great influence in the ARC and in other quarters to fund his personal researches. This was undoubtedly a

mistake, but given Bennet-Clark's character, it was inevitable.

He was a gentle, kind man with a wry sense of humour and a charming eccentricity which endeared him to the hearts of his students and colleagues. He always found time to help those in difficulty and his advice on both scientific and personal matters was appreciated by many. He was an outstanding teacher and lecturer. He liked to pretend that he knew little taxonomy, but his encyclopaedic mind contained a stock of information on taxonomy and many other topics which were subject to instant recall and association in novel ways.

Bennet-Clark contributed greatly to the success of the Society for Experimental Biology and the Journal of Experimental Botany, of which he was the first editor. His scientific achievements were recognised by his election to the Royal Society in 1950 and his work for the Agricultural Research Council was recognised by the award of a C.B.E. The association of the Food Research Institute and the John Innes Institute with the University of East Anglia is a monument to Bennet-Clark. But his colleagues will remember above all his great personal qualities. We loved him and mourn his passing.

D. Davies

announcements

International meetings

March 29–April 2, **Atomic and molecular physics conference**, Belfast (The meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1 8QX, UK).

March 31–April 2, **Intermediate moisture food**, Weybridge, Surrey (The Secretary, National College of Food Technology, St George's Avenue, Weybridge, Surrey KT13 0DE, UK).

Reports and publications

Other countries

The Politics and Responsibility of the North American Breadbasket. By Lester R. Brown. (Worldwatch Paper No. 2.) Pp. 43. (Washington, DC: Worldwatch Institute, 1776 Massachusetts Avenue, NW, 1975.) [1011]

Mass Emergencies, Vol. 1, No. 1, October 1975. Edited by J. Nehnevajsa and E. Quarantelli. (An International Journal of Theory, Planning and Prac-

tice.) Pp. 1–86. Published quarterly Annual subscription Dfl. 86; \$36.75. (Amsterdam: Elsevier Scientific Publishing Company, 1975.) [101]

History of Antarctic Exploration and Scientific Investigation. Plates and Bibliography Compiled by the American Geographical Society. (Antarctic Map Folio Series. Folio 19.) Pp. 6+15 plates. (New York: American Geographical Society, 1975.) \$15 plus \$1 postage and handling. [1011]

United States Department of the Interior: Geological Survey. Professional Paper 763: Stratigraphy of the Inyan Kara Group and Localisation of Uranium Deposits, Southern Black Hills, South Dakota and Wyoming. By Garland B. Gott, Don E. Wolcott and C. Gilbert Bowles. Pp. iv+57+4 plates. (Washington, DC: Government Printing Office, 1974.) \$5.10 [1111]

United States Department of Agriculture. Index-Catalogue of Medical and Veterinary. Supplement 20, Part 1, Authors—A to Z. By Judith H. Shaw, et al. Pp. xvi+558. (Washington, DC: Government Printing Office, 1975.) \$6.15. [1211]

United States Department of the Interior: Geological Survey. Professional Paper 456-1: Geology of the Arabian Peninsula, Jordan. By Friedrich Bender. Pp. vi+36+3 plates. Professional Paper 835: Quaternary Geology of Alaska. By Troy L. Péwé. Pp. v+145+plate 1. Professional Paper 876: Cauldron Subsidence of Oligocene Age at Mount Lewis, Northern Shoshone Range, Nevada. By Chester T. Wrucke and Miles L. Silberman. Pp. iii+20. (Washington, DC: Government Printing Office, 1975.) [1311]

Mauritius Sugar Industry Research Institute. Annual Report for 1974. Pp. 76+15 statistical tables. (Reduit: Mauritius Sugar Industry Research Institute, 1975.) [1411]

United States Department of the Interior: Geo-

logical Survey. Professional Paper 918: Lithium in Unconsolidated Sediments and Plants of the Basin and Range Province, Southern California and Nevada. By Helen L. Cannon, Thelma F. Harms and J. C. Hamilton. Pp. iii+23. (Washington, DC: Government Printing Office, 1975.) [1411]

United States Department of the Interior: Geological Survey. Bulletin 1384: Aeromagnetic and Limited Gravity Studies and Generalized Geology of the Bodie Hills Region, Nevada and California. By F. J. Kelihampl, W. E. Davis, M. L. Silberman, C. W. Chesterman, R. H. Chapman and C. H. Gray, Jr. Pp. iv+38; plate 1. Bulletin 1385-C: Mineral Resources of the San Pedro Parks Wilderness and Vicinity, Rio Arriba and Sandoval Counties, New Mexico. By Elmer S. Santor, Robert B. Hall and Robert C. Weisner. Pp. vi+29+plate 1. Bulletin 1391-C: Mineral Resources of the Clear Creek-Upper Big Deer Creek Study Area, Contiguous to the Idaho Primitive Area, Lemhi County, Idaho. By Fred W. Cater, Darrell M. Pinckney and Ronald B. Stotelmeyer. Pp. vi+41. Bulletin 1394-E: The Auld Lang Syne Group, of Late Triassic and Jurassic(?) Age, North-Central Nevada. By D. B. Burke and N. J. Silberling. Pp. iii+14, 35 cents. Professional Paper 851: Soil Slips, Debris Flows, and Rainstorms in the Santa Monica Mountains and Vicinity, Southern California. By Russell H. Campbell. Pp. iv+51. Professional Paper 871: Lithology and Origin of Middle Ordovician Calcareous Mudmound at Meiklejohn Peak, Southern Nevada. By Reuben James Ross, Jr., Valdar Jaanusson and Irving Friedman. Pp. iv+48. Professional Paper 886: Post-Carboniferous Stratigraphy, Northeastern Alaska. By R. L. Dettmerman, H. N. Reiser, W. P. Brosge and J. R. Dutro, Jr. Pp. iii+46. (Washington, DC: Government Printing Office, 1975.) [1711]